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THE THOMPSON YATES LABORATORIES REPORT

EDITED BY

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WITH THE CO-OPERATION OF

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ERRATA

P. 179. Table III, col. 2, l. 1. Read 68·8 for 18·8.

P. 202, l. 14. Instead of Manson and Thorburn, read *Thurburn Manson and George Warren.*

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THE DISTRIBUTION OF B. COLI COMMUNE

By HARRIETTE CHICK, B.Sc., 1851 Exhibition Scholar

PART II

THE following experiments are a continuation of those described in a previous paper (*T. Y. Reports, Vol. III., p.* 1), and will, I hope, serve to render them more complete. They were undertaken in order, if possible, to answer with more certainty the question whether substances containing B. coli might be considered to be polluted or not, and the only answer to such a question seemed to be a further study of the distribution of the bacillus in question.

The following material has been carefully studied in addition to that already described :—

1 Air.
2 Cultivated manured land.
3 Road dust and sweepings, as well as road puddles.

The experiments substantiate the view I have already put forward, and my present opinion is that the occurrence of this organism, as isolated by the method employed, may be looked upon as useful evidence of recent faecal contamination. At the same time, they show very strikingly a characteristic of B. coli, to which I have already drawn attention, viz. :—the very low resistance which it can offer to unfavourable conditions.

I am aware that this opinion is not general among bacteriologists ; the statement of Kruse (*Zeitschrift f. Hyg. u. Infect. XVII, S.* 53) is constantly quoted, viz. :—that bacilli, closely resembling B. coli, are to be found everywhere, in air, soil, and water from every source. Weissenfeld (*Zeitsch. f. Hyg. u. Infect., XXXIII, S.* 78), has lately shown that in 100 samples of waters he found a 'coli-like' organism in 'good' waters as well as 'bad,' if one litre were taken for analysis. Using one cubic centimetre as a standard, I have found B. coli to be absent in that quantity in 'good waters,' and absent in one litre in specially good, filtered, drinking water (*T. Y. Reports, vol. III, pp.* 14 *and* 16). A good sand filtration seems to be effectual in removing the bacillus, retaining it in the top layer. (*See Table VI, T. Y. Reports, vol. III, p.* 15).

Air.—I have endeavoured to find any satisfactory experimental foundation for the statement that B. coli is to be found in the air, and the only description of

such experiment I have found is in a paper by CONCORNOTTI. (*Centralbl. f. Bakt. XXVI, S.* 492). He exposed in various places agar plates that had already been poured, and incubated them ; he then made an emulsion with sterile water of the mixed cultures he obtained, and injected it intravenously into rabbits. In five cases out of forty-six he obtained cultures of B. coli from the organs and heart blood of the dead animal ; he concluded that this bacillus was present in the emulsion injected, and, hence, in the original air examined. The conclusion, however, appears to be quite unwarranted, for the researches of LESAGE and MACAIGNE (*Arch. d. Med. Exp.* 1892, *p.* 250), ACHARD and PHULPIN (*Arch. d. Med. Exp.* 1895, *p.* 25), and notably those of BECO (*Ann. d. l'Inst. Past.* 1895, *p.* 199), have conclusively proved that B. coli, normally found only in the intestines, is in the habit, both just before and also after death, of invading the organs and even the peripheral circulation. It is significant that most of the experiments of the last-named author were also made upon rabbits. Thus the presence of B. coli, as found by CONCORNOTTI, by no means proved that this bacillus had been the cause of death, or that it had been artificially introduced into the circulation.

I have made a careful examination of the air in different places, but only on one occasion have I isolated B. coli, and that was from the air of an ill-ventilated stable.

Soil, Road Dust.—I have never found the bacillus in virgin soil, and in cultivated manured lands, where it had been added artificially, I have also found it to be absent, a fact which I take to be due to the low power of resistance which B. coli possesses. This is also shown by the almost invariable absence of B. coli in dry samples of road dust, while it occurs in quantity in road puddles or in wet samples of dirt taken during rainy weather. This led me to make some special experiments to illustrate this point, and I found, for example (Table IV, b) that a mixture of a B. coli culture with soil, which contained more than 1,000,000 organisms per grm., after seven and a half hours' drying in the sun had all its organisms killed, while a similar sample, which was kept damp, had its numbers lowered only to 380,000 per grm. These experiments are fully described at the end of the paper, and indicate a conclusion that the presence of B. coli not only is significant of pollution, but shows that the pollution must have been recent.

The method used for these experiments has been already described in detail, viz. : -plating the sample with carbolized agar, incubating for forty-eight hours at 42° C., counting the colonies which resemble B. coli, if present, and isolating and identifying them. For identification I have found the coagulation of milk with the production of acid and the fermentation of lactose the two most distinguishing reactions of B. coli.* A medium containing the latter is conveniently made by simply adding to tap water 2% of peptone and 1% pure lactose ; this is distributed in Durham's

* Harvey Cushing, *Johns Hopkins Hospital Bulletin*, July, 1900.

fermentation tubes, and usually contains no other sugar but lactose. It should, however, always be tested with a culture of B. enteritidis (Gärtner), for example, to see if traces of glucose are present. An organism which, while generally resembling B. coli, coagulates milk and ferments lactose may, I think, be considered to belong to the coli group.

The fermentation of saccharose does not appear to be a constant quality in the group; while ESCHERICH's original B. coli commune, of which I tested a culture, gave no fermentation in a saccharose broth, many isolated from different sources showed production both of acid and gas, while otherwise indistinguishable from the original ESCHERICH culture. For example, out of thirty-seven examples that were thus examined, seventeen fermented saccharose with production of acid and gas, two produced acid but no gas, while sixteen showed no change at all in the medium. The duration of the experiments was from eight to eleven days.

I AIR

The experiments with air, already described,* being somewhat inadequate, the following were undertaken with a view to making the investigation more complete. The original apparatus was discarded in favour of one which was both more convenient and more accurate. As before, a measured quantity of air was drawn through a wool plug, the plug well washed with water, and a portion of the latter plated, and so analysed. As a rule the plug consisted of two parts; the air first went through a glass wool plug and then through one of cotton wool, the former being much easier to wash thoroughly, while the latter is a more efficient filter. The air was drawn through by means of a pump, which was worked by a hot air engine, and each revolution of the engine wheel was recorded by a cyclometer. The record of the cyclometer was calibrated by aspirating a known volume of air, and it was found to give concordant results; the apparatus was usually left running for about twenty-four hours. I am much indebted for the use of the machine to Dr. E. E. GLYNN, under whose direction the whole apparatus was put together.

Experiment I—The air outside the laboratory was analysed on the night of April 30-31, 1900, after wet weather. B. coli was found absent in 240 litres; a bacillus resembling B. fœcalis alealigenes was, however, isolated on this occasion. The details of this and of all the other experiments are given in Table I.

Experiment II—The air of the College grounds was analysed for five consecutive days, in dry, dusty weather, from May 14 to May 19, 1900. B. coli was found invariably to be absent.

Experiment III—A dusty street corner was chosen as the place for analysis of the air, which was examined for seven consecutive days; the air was drawn

* *T. Y. Reports*, vol. III, p. 11.

through continuously, the plug being removed each day for analysis. B. coli was absent in every analysis.

Experiment IV—In this experiment the air analysed was that of two of the wards of the Royal Infirmary, and B. coli was also found to be absent.

Experiment V—The air was that of an ill-ventilated stable, and was examined on three occasions ; on the first B. coli was isolated and found present in 238 litres. The air was drawn through during the night in each instance, and the plug analysed early on the following morning.

TABLE I

Experiment I—Air of College Grounds

Date	Quantity of air aspirated	Quantity analysed	Remarks
1900 April 30-31	596 litres	B. coli absent in 240 litres	Rain on 29 Bacillus closely resembling B. fœcalis alcaligenes isolated

Experiment II—Air of College Grounds

May 14-15	3,570 litres	B. coli absent in 890 litres	No rain since May 12
May 15-16	2,580 litres	B. coli absent in 646 litres	No rain since May 12
May 16-17	3,960 litres	B. coli absent in 1,950 litres	No rain since May 12, days from May 16-18 were dry and dusty, and much dust was blowing about
May 17-18	1,790 litres	B. coli absent in 880 litres	
May 18-19	3,130 litres	B. coli absent in 620 litres	

Experiment III—Air at the Corner of Brownlow Hill and Ashton Street Liverpool

May 24-25	3,856 litres	B. coli absent in 1,930 litres	Machine calibrated May 24 ; rain on May 23 ; May 25, a dry, dusty day
May 25-26	3,670 litres	B. coli absent in 1,840 litres	May 26, windy, dusty day
May 26-28	12,400 litres	B. coli absent in 4,135 litres	
May 28-30	7,650 litres	B. coli absent in 3,826 litres	
May 30-31	4,520 litres*	B. coli absent in 2,260 litres	

* At the conclusion of Experiment III, it was found that the washer of the piston of the pump was slightly worn out, so that at the end of the experiment the registered quantity of air may be a little too large, the friction of the machine being less. A calibration after a new washer had been fitted gave the same number as on May 24.

EXPERIMENT IV—AIR OF THE ROYAL INFIRMARY, LIVERPOOL

Date	Quantity of air aspirated	Quantity analysed	Remarks
June 8th.	856 litres	B. coli absent in 430 litres	Ward I (Medical), well ventilated
June 11th	2,000 litres	B. coli absent in 1,000 litres	Ward XII (Medical), well ventilated

EXPERIMENT V—AIR OF A STABLE

July 11-12	2,380 litres	B. coli present in 240 litres	Stable badly ventilated.
July 12-13	2,480 litres	B. coli absent in 743 litres	Stable badly ventilated.
July 17-18	1,600 litres	B. coli absent in 800 litres	Stable badly ventilated.

II CULTIVATED SOILS (MANURED)

Samples of ploughed land were taken in the neighbourhood of Shrewsbury on June 23 and June 24, 1900; the weather was rainy, and most of the samples were damp, both when taken and when analysed. As will be seen from Table II, B. coli, which must have been added in quantity to the land when manured, was absent in almost every case in the quantity analysed. In No. 3, where B. coli was present, the sample was taken from a garden close to the shore of the river Severn, where the ground would probably be always kept moist. The samples were taken about an inch below the surface, as a rule, and were all in neighbourhoods which drain into the river Severn; this is interesting in connection with the analyses of a number of land drains running into that river (*T. Y. Reports, vol. III, p.* 25), in which B. coli was also found to be absent. The crops were only just showing, so that the time of manuring was probably not very far distant, but the B. coli had apparently been unable to withstand the drying, &c., it had undergone in the meantime.

TABLE II

Cultivated Soil

No.	Description of the Field, &c.	B. Coli Commune, per grm.	Remarks
1	Potato field, Buildwas	Absent in ·02 grm.	Samples taken June 23, and plated June 25, 1900 (Wet weather)
2	Same field, another place	Absent in ·01 grm.	Samples taken June 23, and plated June 25, 1900 (Wet weather)
3	Garden, growing turnips, Buildwas, on the shore of the river Severn	205 per grm. (approx.)	Samples taken June 23, and plated June 25, 1900 (Wet weather)
4	Ploughed field, crops just growing	Absent in ·02 grm.	Samples 4-13 taken on June 24, and plated on June 26 (Wet weather)
5	Ploughed field, turnips	Absent in ·03 grm.	Sample dry when analysed
6	Ploughed field, crops just growing	Absent in ·02 grm.	Sample dry when analysed
7	Ploughed field	Absent in ·02 grm.	Sample wet when analysed
8	Ploughed field	Absent in ·02 grm.	Sample dry when analysed
9	Ploughed field, crops just growing	Absent in ·006 grm.	Sample dry when analysed
10	Ploughed field, Pitchford	Absent in ·025 grm.	Sample dry when analysed
11	Ploughed field, cabbages, crops very small	Absent in ·04 grm.	Sample wet when analysed
12	Ploughed field, crops just growing	Absent in ·02 grm.	Sample dry when analysed
13	Ploughed field, crops just growing	Absent in ·02 grm.	Sample wet when analysed
14	Garden, Grange-over-Sands, July 8, 1900	Absent in ·04 grm.	Sample was taken from the base of a rose-bush, where there were obvious remains of manure ; a damp sample

III ROAD DUST, &c.

In all thirty-three samples of road dust and puddles were examined, the latter, and those of the former taken during wet weather, usually contained B. coli in quantity, for example, those examined on May 7 (*see Table III*, where the numbers must be considered as approximate only). The dirt was collected in sterile test tubes, weighed out into sterile flasks, the requisite amount of sterile water added for the required dilution, the whole well shaken, and 1 c.c. plated on carbolized agar. This method has been the one ordinarily followed, and the preceding samples of soil were analysed exactly in the same way. Dry samples, as a rule, contained no B. coli in the quantities analysed, and it is significant that when among a number of dry samples, wet ones were examined on the same day, the latter invariably contained B. coli (*e.g.*, *No.* 18,

Table III). The dampness may have been caused by artificial watering of the street in question, or by the constant trickling of water from a fish shop serving to keep a gutter moist, as in No. 14.

Whether a moist substratum would serve to resuscitate B. coli, which had apparently died out, or whether in such dry samples the bacillus was quite dead, was answered in the following manner. The dilutions of May 31 (*see Nos.* 26-31, *Table III*) were kept for three days and then analysed again. The results showed that in the cases in which B. coli was absent on May 31, it was also absent after the sample had been thus kept wet and favourable for the growth of the bacillus; in those cases in which it was present on May 31, its numbers were much reduced on June 2. One must conclude that B. coli, constantly being thrown upon the roads, is able to survive in dry weather for a short time only (*No.* 33); if, however, the weather is wet or the road kept damp artificially, it can live much longer.

These facts suggested the enquiry whether sunshine or drying was the important factor in the short life of B. coli in nature under these conditions. The effect of drying has been studied by WALLIEZEK (*Centralbl. f. Bakt. XV, S.* 949), in whose experiments 17 hours' exposure to drying over sulphuric acid killed almost all the bacteria on a piece of filter paper that had been dipped in a culture of B. coli and then dried; a similar effect was obtained after 45 minutes' drying in a vacuum. BILLINGS and PECKHAM (*Science, Feb.*, 1895, *Abstr. Centralbl. f. Bakt. XIX, S.* 244), found that threads moistened with a broth culture of B. coli and then dried, showed living bacteria for a period of 152-229 days, while sunlight alone proved fatal in 3-6 hours; they concluded that death by sunlight is on no account to be attributed to drying. BUCHNER (*Centralbl. f. Bakt. XI, S.* 781), also showed the importance of sunlight in killing B. coli; in one experiment 100,000 B. coli suspended in water and exposed to sunlight were all found dead after one hour.

The experiments of WITTLIN (*Wiener Klin. Wochenschr.*, 1896, *No.* 52, *S.* 1,229), however, indicate that drying must be a factor in the death of B. coli in nature, as important as sunshine, if not more so; and the experiments I am about to describe confirm his observations.

An emulsion was made with a twenty-four hours' culture of B. coli on agar and a little water, and this was used to moisten some sterilised earth, which was then distributed into six Petri dishes. The soil in all of these was analysed for B. coli in the way already described; in the subsequent analyses, where the earth was really wet, it was dried before weighing on sterile filter paper. This method, I think, makes the numbers of the wet samples rather lower than they should be, and renders the differences a little less striking than they really are. After analysis, water was added to three of the six samples, and these were kept thoroughly wet throughout the course of the experiment, the other three were allowed to dry. One of each was

placed in the sunshine, two also in the shade, and two were kept in the dark. The details are given in Table IV ; after exposure to six hours' sunshine, both wet and dry samples contained no B. coli in the quantities analysed ; of the shade samples, the wet one contained 247,000 per grm., while in the dry sample there was no B. coli. No 5 was scarcely dry by the evening of the first day ; on the next day, however, it also contained no B. coli. The two samples 5 and 6 were then left for nearly two months, at the end of that time, while No. 5 was sterile as regards B. coli, No. 6, which had been kept moist all the time, contained 566,000 per grm. Though at the commencement of the experiments care was taken to exclude all other organisms by sterilising, no attempt was made to keep the contents of the dishes sterile afterwards, thus attempting to approximate to the conditions which obtain in nature.

Experiment II—(*Table IV*, *b*), consisting of two samples only, a wet and a dry one, both exposed to sunshine, was made to correct a discrepancy in Experiment I. After the analysis on July 27, the two Petri dishes with their contents were placed in a dark cupboard, and after nearly two months were again analysed. The result was the same as in Experiment I ; while in the dry sample B. coli was absent, in the wet one it was present to the extent of 47,000 per grm.

Experiment III was made when there was not so much sunshine, and that rather intermittent ; it, however, also illustrates the marked difference in the length of life of B. coli under dry and wet conditions.

Some experiments were also made with a pure culture of B. coli in order to find its length of life when dried upon filter paper. Scraps of sterile filter paper, about an inch square, were dipped into a 'peptone-salt' culture, twenty-four hours old, of B. coli, and these were drained and then laid upon a sterile Petri dish. The pieces of paper were just the same size, and hence retained approximately the same number of organisms. They were placed under a tightly-fitting bell-jar, and a current of dry air was drawn over them by means of a pump, so that they were quickly dried. From time to time, one of these pieces of paper was removed and incubated with 10 c.c. nutrient broth ; after twenty-four hours plates of ordinary nutrient agar were made of 1c.c. of the broth and these examined, after forty-eight hours' incubation, to see if they were sterile. It was found that there had remained no B. coli in the piece of paper which had been dried for twenty-eight hours, while B. coli was almost entirely killed after twenty-three and a quarter hours exposure to the current of dry air. A culture of B. coli commune from the original strain of ESCHERICH was used for these experiments, kindly given me by Dr. H. E. DURHAM. *Experiment II* gave a similar result ; after twenty-four hours' exposure of the pieces of paper to a current of dry air, all the organisms were found to have been killed. An interesting series of plates can be obtained by taking the dried pieces of filter paper at different times, moistening them with sterile water and pressing them on the surface of an already poured agar plate for a few minutes ; then the paper should be

removed and the plate incubated. At first the shape of the paper is shown after incubation as a solid mass of growth, after six hours drying (*No.* 4, *Table V*, *Exp. II*) there were only a few scattered colonies, and after ten hours drying (*No.* 5, *Exp. II*) the patch remained quite sterile. This test, of course, is not so searching as incubating the paper with broth for twenty-four hours and then plating the broth culture.

This makes it easy to understand how it is that one frequently does not find any trace of the B. coli in places where it must undoubtedly have been present in quantity not so very long before, *e.g.*, in roads, manured land, &c. The effect of drying, shown in the above experiments, would account for its absence. A question of practical interest arising out of this is whether the streets should be watered or not in the dry weather. MAZUSCHITA (*Archiv. f. Hygiene*, 1899, 35, *S.* 252), found in Freiburg that the life of certain bacteria in watered dust was three times as long as in dry dust, and also that on an average there were twice as many organisms in the dust of a watered street as in that of one which was unwatered. He considers that although the watering of streets lengthens the life of the bacteria in the dust, that it is still useful in laying it, and thus keeping it away from the respiratory organs ; as an improvement he would suggest thoroughly flushing the streets at frequent intervals.

In conclusion, I wish again to give my best thanks to Professor BOYCE, at whose suggestion the work was originally undertaken, and to whom I am indebted for much kind help and advice during the course of the investigation.

TABLE III

No.	Date	Description and Source of Sample	Dilution used	Presence or Absence B. coli in quantity analysed	Remarks
	1900				
1	May 1			Absent in .2 grm.	Dry weather
2	„ 1	Dust of roads		„ .16 „	Dry weather
3	„ 1			„ .15 „	Dry weather
4	„ 1			„ .15 „	Dry weather
5	„ 7	Dirt, side of road, Bedford St., L'pool.		*65,000 per grm.	Damp weather
6	„ 7	„ „ Walnut Street, „		* 1,200 „ „	Damp weather
7	„ 7	Puddle water, Ashton Street, „	$\frac{1}{50}$	500 „ c.c.	Damp weather
8	„ 7	„ „ Huskisson Street, „	$\frac{1}{50}$	* 4,200 „ c.c.	Damp weather
9	„ 7	„ „ Bedford Street, „	$\frac{1}{50}$	750 „ c.c.	Damp weather
10	„ 7	„ „ „ „ „	Neat	25 „ c.c.	Damp weather
11	„ 15	Dirt, middle of road, Edge Lane, „	.2 grm. in 50 c.c.	Abs. in .012 grm.	Dry sample
12	„ 15	„ side of road, „ „ „	1.18 „ „ 50 c.c.	Abs. in .07 „	Dry sample
13	„ 15	„ „ Marmaduke Street, „	.98 „ „ 50 c.c.	Abs. in .06 „	Dry sample

TABLE III—*continued*

No.	Date	Description and Source of Sample	Dilution used	Presence or Absence B. coli in quantity analysed	Remarks
14	1900 May 15	Dirt, side of Paddington, L'pool.	1.05grm.in 100c.c.	* 4,400 per grm.	Damp sample, taken at side of road, opposite a fish shop, by no means clean, where the gutter is always wet
15	„ 15	„ „ Brownlow Hill, „	1.2 „ „ 50c.c.	Probably absent in .07 grm.	Dry sample
16	„ 17	„ Crosshall Street, „	1.04 „ „ Water 100c.c.	Abs. in .03 „	No rain since May 12. May 17 a dry, dusty day; dry sample
17	„ 17	„ Victoria Street, „	.65 „ „ 100c.c.	Abs. in .02 „	Dry sample
18	„ 17	„ St. John's Lane, „	1.91 „ „ Water 100c.c.	1,200 (at least) per grm.	Damp sample
19	„ 17	„ South Gate, St. George's Hall, „	1.05 „ „ Water 100c.c.	Abs. in .06 „	Dry sample
20	„ 17	„ London Road, Seymour St., „	.82 „ „ 50c.c.	Abs. in .05 „	Dry sample
21	„ 17	„ Seymour Street, „	.16 „ „ 20c.c.	Abs. in .02 „	Dry sample
22	„ 17	„ Russell Street, „	.53 „ „ 100c.c.	Abs. in .016 „	Dry sample
23	„ 17	„ Brownlow Street, „	.85 „ „ 100c.c.	Abs. in .025 „	Dry sample
24	„ 31	„ Ling Street „	.49 „ „ 100c.c.	Abs. in .03 „	Dry sample
25	„ 31	„ Brae Street „	1.51 „ „ 100c.c.	Abs. in .045 „	Dry sample
26	„ 31	„ Kensington Park, „	.94 „ „ 50c.c.	(*a*) Abs. in .06 „	Dry sample
„ (*b*)	June 2	„ „ „ „	.94 „ „ 50c.c.	(*b*) Abs. in .06 „	Dry sample
27	May 31	„ Jubilee Drive, „	.49 „ „ 50c.c.	*(*a*) 29,900 per grm	Wet sample
„ (*b*)	June 2	„ „ „ „	.49 „ „ 50c.c.	†(*b*) 2,350 „ „	Fewer total organisms and many fewer B. coli than on May 31
28	May 31	„ Edge Lane, „	1.07 „ „ 50c.c.	*(*a*) 2,100 „ „	Wet sample
„ (*b*)	June 2	„ „ „ „	1.07 „ „ 50c.c.	†(*b*) 327 „ „	More total organisms, but not so many B. coli
29	May 31	„ Marmaduke Street, „	.87 „ „ 100c c.	*(*a*) 340 „ „	Wet sample
„ (*b*)	June 2	„ „ „ „	.87 „ „ 100c.c.	(*b*) abs. in .001 grm	
30	May 31	„ Brownlow Hill, „	.55 „ „ 50c.c.	(*a*) abs. in .03 „	Dry sample
„ (*b*)	June 2	„ „ „ „	.55 „ „ 50c.c	(*b*) abs. in .03 „	
31	May 31	„ College Grounds, University College, L'pool.	.71 „ „ 50c.c.	(*a*) abs. in .04 „	Dry sample
„ (*b*)	June 2	„ „ „ „ „	.71 „ „ 50c.c.	(*b*) abs. in .04 „	
32	July 7	Dust, middle of road, Grasmere	1 in 100	Abs. in .04 „	Dry sample (Weather July 6, very wet)
33	„ 8	„ „ „ Grange-over-Sands	1 in 100	3,900 per grm.	Dry sample

* Numbers are only approximate. † These numbers are a maximum. The dilutions of May 31 kept and plated again.

TABLE IV (A)

EXPERIMENT I

	July 24, 1900, 11-45 a.m., beginning of Expt. B. coli per grm.	July 24, 5-45 p.m., after 6 hours' exposure to sun in case of 1 and 2. B. coli per grm.	July 25, 6 p.m., after 6½ hours' exposure to bright sun in case of 1 & 2. B. coli per grm.	July 27, 12 p.m. B. coli per grm.	Sept. 21, Nos. 5 and 6 kept in dark since July 27. B. coli per grm.
Contents of Petri Dish—					
No. 1 (exposed to sun and allowed to dry)	1,210,000	Absent in .24 grm.	Absent in .5 grm.		
No. 2 (exposed to sun and kept wet)	592,400	Absent in .008 grm.	‡1,170		
No. 3 (in shade and allowed to dry)	850,000	Absent in .04 grm.	Absent in .6 grm.		
No. 4 (in shade and kept wet)	336,000	247,000	3,360,000		
No. 5 (in dark and allowed to dry)	390,000	1,020,000*	Absent in .02 grm.	Absent in .5 grm.	†Absent in .24 grm.
No. 6 (in dark and kept wet)	508,500	56,800	1,870,000	1,180,000	566,000

* Was not then properly dry. † It was impossible to take more for analysis on account of the extraneous organism which had found their way in. ‡ There appears to be a discrepancy here, to correct which Experiment II was undertaken.

TABLE IV (B)

EXPERIMENT II

	July 26, 1900, 10-15 a.m. B. coli per grm.	July 26, 5-45 p.m., after 7½ hours' sunshine. B. coli per grm.	July 27, 12 noon, after 5½ hours' sunshine. B. coli per grm.	Sept. 21, in dark since July 27. B. coli per grm.
Petri-dish—				
No. 7 (exposed to sun and allowed to dry)	1,050,000	Absent in .13 grm.	Absent in .5 grm.	†Absent in .28 grm.
No. 8 (exposed to sun and kept wet)	1,220,000	381,000	1,584,000	47,000

† It was impossible to take more for analysis, owing to the extraneous organisms which had found their way in.

TABLE IV (c)

EXPERIMENT III

	Sept. 25, 1900, 10-15 a.m. B. coli per grm.	Sept. 25, 1-50 p.m., after 3½ hours' intermittent sunshine. B. coli per grm.	Sept. 25, 5 p.m., after 4¾ hours' intermittent sunshine. B. coli per grm.	Sept. 26, 5 p.m., no sun all day. B. coli per grm.	Sept. 28, after 6¾ hours' sunshine. B. coli per grm.	Oct. 6, C. and D. after about 38 hours' light. B. coli per grm.	Oct. 22, D had exposure to 55 hours light. B. coli per grm.
Petri-dish—							
(A) Exposed to sunlight, and allowed to dry	3,800,000	42	9	Absent in .3 grm.			
(B) Exposed to sunlight, and kept wet	4,250,000	1,015,000	547,000	354,000	1,090,000	plate broken	
(C) In the shade, and allowed to dry	2,250,000		Absent in .03 grm.	5	Absent in ·3 grm.	Absent in ·3 grm.	
(D) In the shade, and kept wet	3,640,000		1,030,000	1,450,300	1,982,000	800,000	82,500
(E) In the dark, and allowed to dry	4,300,000		420	7	Absent in .3 grm.	Absent in .6 grm.	
(F) In the dark, and kept wet	3,420,000		405,000	1,650,000	1,912,000	1,600,000	166,000

TABLE V (A)

EXPERIMENT I

No.	Time	No. of hours' exposure to current of dry air	Appearance on plate (piece of paper incubated for 24 hours with 10 c.c. broth and 1 c.c. plated).
1	Oct. 1, 1900, 10-55 a.m.	0	∞ Colonies
2	" " 12-45 noon	2	∞ "
3	" " 2-55 p.m.	4	∞ "
4	" " 5-10 p.m.	6¼	∞ "
5	" 2, " 10 a.m.	23¼	9 "
6	" " 2-45 p.m.	28	Sterile
7	" 3, " 9-40 a.m.	47¾	Sterile

TABLE V (B)

EXPERIMENT II

No.	Time	No. of hours' exposure to current of dry air	Appearance on plate (piece of paper incubated for 24 hours with 10 c.c. broth and 1 c.c. plated).
1	October 4, 10-40 a.m.	0	∞ Colonies
2	„ „ 12 noon	1⅓	∞ „
3	„ „ 2-30 p.m.	4	∞ „
4	„ „ 4-30 p.m.	6	∞ „
5	„ „ 8-45 p.m.	10	∞ „
6	„ 5, 10-35 a.m.	24	Sterile

APPENDIX—TABLE VI

This table shows the efficiency of sand filtration for removing B. coli commune from water *(see this vol., pp. 15 and 16)*. The samples were taken from a sand filter bed while it was being cleaned. The bed was used to filter the Rivington supply of the Liverpool drinking water.

Date	No.	Description of Sample	B. coli per gram	Remarks
Sample taken July 2, 1900, analysed July 3, 1900	1	Sand from unscraped surface of filter bed	20	Sand appeared very dirty
Sample taken July 2, 1900, analysed July 3, 1900	2	Sand from same bed, after the surface (about ½ inch deep) had been scraped off	Absent in ·3 grm.	
Sample taken July 2, 1900, analysed July 3, 1900	3	Sand, similar to No. 1, after washing	„ „	

THE RELATION BETWEEN BACILLUS ENTERITIDIS SPOROGENES OF KLEIN AND DIARRHOEA*

By E. E. GLYNN, M.A., M.B.

The bacillus enteritidis sporogenes was discovered by Dr. KLEIN during certain investigations concerning the cause of an epidemic of diarrhoea. The following is an abstract of the epidemic taken from the report by Dr. ANDREWES, Health Officer to St. Bartholomew's Hospital.† 'On the night of October 27, 1895, a sudden outbreak of diarrhoea of a severe type occurred amongst the in-patients of St. Bartholomew's Hospital. Out of twenty-eight wards fifteen were affected. The total number of cases during the night and following morning was fifty-nine. The outbreak ceased as suddenly as it began, and by mid-day on October 28 it was practically over.' Clinically, the earliest symptom in nearly all cases was abdominal pain. 'This was followed in the course of half an hour or more by diarrhoea. Vomiting was conspicuously absent in the great majority of the cases. The disorder was intestinal, not gastro-intestinal. In some instances the bowels were opened six or eight times in the course of twelve hours, but in the majority of cases only two or three times. The stools were in most cases liquid and watery, and a considerable amount of mucous was present. In the more severe cases a considerable degree of prostration occurred. But in no case was the attack fatal, even when the patient had been previously suffering from severe disease.'

On microscopical examination of the evacuations and also of the mucous discharge, a very large number of oval glistening spores were detected. These, Dr. KLEIN demonstrated by anaerobic cultivation, were derived from a hitherto unknown, and 'uncommon,'‡ organism, the bacillus enteritidis sporogenes. Moreover, milk cultures of this bacillus inoculated subcutaneously into guinea-pigs led to severe haemorrhagic oedema with gangrene, and death in about twenty-four hours.

The main features of the outbreak pointed to 'the toxic character of some article of diet supplied to the patients alone,' for the nurses, with one exception, escaped. By process of exclusion the milk was suspected. Since none of this incriminated milk was available for bacteriological examination a sample 'taken from the same source as that supplied on October 27 was furnished by the company

* Part of this article was presented to the University of Cambridge as a thesis for the degree of Bachelor of Medicine.
† *Medical Officer's Report*, Local Government Board, for 1895-96, p. 197-198.
‡ *Medical Officer's Report*, Local Government Board, for 1895-96, p. 204.

supplying the hospital, on October 30.' This sample contained the spores of bacillus enteritidis, and the cultures were virulent to guinea-pigs. 'Inquiry into the source of the milk of the company which supplied it, elicited the facts that on Sunday, October 27, seventeen gallons out of a total of 190 gallons came from a certain farm at Melton Mowbray, and the sample furnished on October 30 was from the same farm.' Dr. ANDREWES also remarks, 'that on the supposition that the milk was the cause of the diarrhoea outbreak, the irregular and capricious distribution of the disease in the hospital need excite no surprise, for the manner in which the milk was sent out to the different wards would well explain it. It was poured through a strainer into a wooden tub, from which the ward vessels were filled, and this tub was never allowed to get empty, but a fresh churn was poured into it when it got low, thus leading to an irregular mixture of the milk from one churn with that of the others.'*

It was concluded from these observations that the outbreak in question 'appeared referable'† to the bacillus enteritidis sporogenes, 'a diarrhoeal organism.'‡

I have attempted to state as clearly as possible what seem to be the main reasons for this conclusion—

(1) The spores of bacillus enteritidis were demonstrated in the evacuations of the persons attacked.

(2) The outbreak was attributed to milk from a certain farm, and it was assumed, inasmuch as the spores of the bacillus enteritidis were detected in a sample of milk obtained from this farm three days after the outbreak, that they were also present in the original toxic milk.

(3) The bacillus enteritidis was regarded as an 'uncommon' organism, and had not been detected in the normal evacuations of healthy individuals.

(4) The milk cultures obtained from the evacuations and from the hospital milk were pathogenic to guinea-pigs.

It is curious that milk obtained from the Melton Mowbray farm 'was supplied both before and since the outbreak' at St. Bartholomew's 'to other customers without producing any harmful effect, so far as the company's knowledge went.'§

At present B. enteritidis can only be detected in a given material by cultivating its spores, consequently if the bacillus is present in a non-sporing condition, the result of cultivation will be negative. However, as B. enteritidis very readily forms spores, their absence must be taken, in practice, to indicate the absence of the bacilli also.

In order to isolate the spores from faeces, a small particle is inoculated into a tube of *recently* sterilised litmus milk, which is heated for fifteen minutes from 70°C.

* *Medical Officer's Report*, Local Government Board, for 1895-96, p. 198.
† *Medical Officer's Report*, Local Government Board, for 1895-96, p. 197.
‡ *Medical Officer's Report*, Local Government Board, for 1895-96, p. 204.
§ *Medical Officer's Report*, Local Government Board, for 1895-96, p. 198.

up to 80°C. to kill non-sporing organisms. The tube is then cooled and placed with a certain quantity of a fresh solution of pyrogallic acid and caustic potash in a BUCHNER's cylinder, tightly corked, and incubated at 37°C. In from twenty-four to forty-eight hours, if the bacillus is present, a most striking and characteristic change occurs. The milk is 'curdled with copious evolution of gas, the spongy curd floating in a fairly clear transparent whey;' the reaction is acid, with a smell of butyric acid. Microscopical examination reveals multitudes of bacilli in the form of 'rods and cylinders,' which stain by the method of GRAM.

The virulence of the organism is classed as normal by Dr. KLEIN, if the subcutaneous inoculation of one cubic centimetre of whey from a recently changed milk culture into a guinea-pig weighing from 200-300 grammes is fatal in about thirty hours or less, the animal dying with 'severe haemorrhagic oedema and gangrene of the subcutaneous tissues, spreading widely from the point of inoculation.' The virulence of the organism is diminished if the guinea-pig dies slowly, or recovers with extensive sloughing of the skin in the neighbourhood of inoculation. Lastly, if the only result of inoculation is a transient swelling without subsequent necrosis, the milk culture contains the bacillus of BOTKIN; an organism which is said to resemble the bacillus enteritidis sporogenes in almost every respect save one, viz., that its cultures are non-pathogenic when inoculated subcutaneously into guinea-pigs or rabbits.

The only other anaerobic sporing organism with which bacillus enteritidis might be confounded is the bacillus of malignant oedema. This organism, as KLEIN points out, does not stain by GRAM's method, and does not produce in anaerobic milk cultures, or when inoculated into guinea-pigs, the changes which are characteristic of bacillus enteritidis sporogenes.* According to KLEIN, the presence of the bacillus enteritidis is proved if an anaerobic cultivation of its spores in milk produce certain reactions, and if the resulting whey exerts certain definite pathogenic effects when inoculated subcutaneously into the groin of a guinea-pig. Microscopical examination of the organism and subcultivation is not necessary.

I have, however, during observations on the spores of bacillus enteritidis obtained from various sources, examined microscopically as a matter of routine a large number of typical milk cultures, and in every instance detected, usually in pure culture, the characteristic bacilli which stained by the method of GRAM.

Subcultivation of bacillus enteritidis, like other anaerobic organisms, on solid media, is unsatisfactory. Although I have obtained in blood serum, glucose agar, and glucose gelatin, pure colonies of the bacillus, the usual result of transplantation into milk is not a 'typical' culture, but one deficient in whey and gas formation, and containing many spores. 'This ability to cause atypical change in milk occurs,' according to KLEIN, 'sooner or later to bacillus enteritidis from any given source.'†

* *Medical Officer's Report*, Local Government Board, 1897-98, pp. 227-229.
† *Medical Officer's Report*, Local Government Board, 1897-98, p. 224.

On the night of Sunday, March 6, 1898, a second epidemic of diarrhoea similar to the first broke out among the in-patients of St. Bartholomew's Hospital, there being 144 severe cases. The milk was proved by Dr. ANDREWES to be the 'vehicle of infection.' On bacteriological examination spores of bacillus enteritidis of a virulent character were demonstrated in the evacuations of the patients affected, and also in a sample of Sunday morning's milk.*

On Friday night, August 5, 1898, the hospital was again attacked, eighty-six persons were affected, the majority of whom had eaten certain rice pudding. Bacillus enteritidis was isolated in a sporing condition from the diarrhoeal evacuations, and in a non-sporing condition from a sample of the suspected rice pudding ;† while Dr. KLEIN remarked, 'the conclusion becomes irresistible that this microbe was the direct cause of the disease.'‡ Clinically, the second and third epidemics resembled the first, and every patient recovered.

Obviously it is important to discover whether a particular organism—the bacillus enteritidis sporogenes—is probably or certainly a cause of diarrhoea.

Now, in the first place, if the existence of enteritidis spores in the evacuations of the patients at St. Bartholomew's can be used as an argument that the bacillus was, or probably was, the cause of the diarrhoea, it is clear that the spores should be almost or entirely absent in the evacuations of healthy persons, and of those suffering from diarrhoea which is casual and commonplace, or secondary to organic disease, as cirrhosis of the liver or typhoid.

Again, as the virulence of B. enteritidis to guinea-pigs is subject to considerable variation, one would expect, even if the spores were constantly present in all evacuations, that their virulence would be considerably less than the virulence of those obtained from the intestines of individuals suffering from diarrhoea directly due to the activity of the microbe.

Therefore it is essential to discover, firstly, whether the spores of enteritidis exist in normal and diarrhoeal evacuations generally ; and secondly, if present, whether they are equally virulent.

In the summer of 1897 Dr. KLEIN made some observations regarding the presence and virulence of bacillus enteritidis in infantile diarrhoea.§ He examined the stools of eleven cases ; in nine the diarrhoea was acute, generally with symptoms of gastro-enteritis ; in the remaining two it was more chronic ; ten of the infants died. It was demonstrated that the spores occurred in a virulent form in four out of the ten fatal cases, while in the remaining six and in the one that recovered, cylindrical rods were discovered on microscopical examination of the bowel contents, which might

* *Medical Officer's Report*, Local Government Board, 1897-98, p. 235.
† *Lancet*, January 7, 1899, p. 8.
‡ *Medical Officer's Report*, Local Government Board, 1898-99, p. 336.
§ *Medical Officer's Report*, Local Government Board, 1897-98, p. 230-233.

have been bacillus enteritidis in a non-sporing state. Further, spores of normal virulence were also isolated from six out of six cases of cholera nostras in adults, three being fatal.*

Now, if bacillus enteritidis produced the infantile diarrhoea, why were the spores apparently absent in the evacuations of seven out of eleven patients? But assuming six cases of cholera nostras and four of infantile diarrhoea were due to this bacillus, why did seven of them die, whereas all the 291 persons attacked at St. Bartholomew's recovered, although at the time several were seriously ill from intercurrent diseases? Why, also, were the cultures of enteritidis obtained from the mild cases which recovered equally virulent to those obtained from the severe cases which died? Indeed, considering the presence or absence of enteritidis spores in the evacuations, some evidence in support of the proposition that the bacillus was the agent producing the St. Bartholomew's epidemics and also, in certain instances, infantile diarrhoea and cholera nostras,† was the fact that Dr. KLEIN believed they were absent in the normal evacuations; a statement which he has since modified.‡

Dr. ANDREWES, in 1896, investigated the anaerobic organisms existing in twenty consecutive cases of diarrhoea.§ From one to three loopfuls of faecal matter were inoculated into grape sugar gelatine, heated between 78° and 80° for ten minutes, and incubated anaerobically at 20° C. The only microbe constantly present was bacillus enteritidis sporogenes, or a 'variety'; it was isolated from twelve individuals. The 'variety,' as Dr. KLEIN pointed out, was most probably the true enteritidis growing atypically, as frequently obtains under certain conditions. I have attempted to summarise Dr. ANDREWES' results. The spores of enteritidis were present in—

(*a*) Four out of ten cases of acute diarrhoea with symptoms, often severe, of intestinal or gastro-intestinal irritation.

(*b*) Four out of six cases of diarrhoea without enteritis.

(*c*) In diarrhoea associated with catarrhal jaundice, also with gastric ulcer.

(*d*) In a case of fatty diarrhoea, and in the watery offensive stool of a child.

The results of inoculation are not stated in six instances; in four the cultures were of diminished virulence, in one non-pathogenic, while an anaerobic milk culture from the evacuations of a man who died with choleraic symptoms was exceedingly virulent.

The explanation of the diminished virulence is the fact that the gelatin cultures, liquefied by the bacillus, were injected, and as the gelatine sometimes liquefied slowly, 'it is probable that some such cultures had lost their virulence.'¶

* *Medical Officer's Report*, Local Government Board, 1897-98, p. 234.
† *Medical Officer's Report*, Local Government Board, 1897-98, p. 211.
‡ *Medical Officer's Report*, Local Government Board, 1898-99, p. 330.
§ *Medical Officer's Report*, Local Government Board, 1896-97, p. 255-62.
¶ *Medical Officer's Report*, Local Government Board, 1896-97, p. 260.

Dr. ANDREWES was led to this conclusion, among others, 'Whether the bacillus in question is actually a cause of acute diarrhoea is not so far positively established, but there seems to be a distinct probability that such is the case.'†

There is, however, in my opinion, one striking feature in these experiments, *i.e.*, the spores were apparently absent in five cases of acute sporadic diarrhoea, three being choleraic; and present in two instances at least, in which the diarrhoea was merely looseness of the bowels without any acute symptoms.

Professor BOYCE suggested to me, during some observations on bacillus coli commune in diarrhoeal evacuations, that I should also ascertain whether the spores of bacillus enteritidis were present. Now, it was necessary for the isolation of bacillus coli, by means of agar plates, to use very dilute solutions of stool; and it was these dilute solutions which were first tested for the spores of enteritidis, and consequently the quantity of stool examined was very minute. The dilution was the following:—One platinum loopful of faecal matter was well mixed with one cubic centimetre of sterile water, and as a rule from one to five loops of this solution were inoculated into litmus milk, subsequently heated for fifteen minutes from 70° C. up to 80° C., and inoculated anaerobically. The size of the platinum loop used on different occasions was approximately the same, for it was made by using a piece of wire as a gauge. I have found by careful measurement that one cubic centimetre of water contains about thirteen hundred such loopfuls, and consequently a rough comparison of the dilution used may be obtained by adopting the following standards:—One loopful of a solution of one loopful of stool in one cubic centimetre of water represents a dilution of 1,300; while if five loopfuls are taken the dilution is 260; if half a cubic centimetre the dilution is two, &c.

The samples of stools obtained from hospital patients were kindly provided in sterilised vessels by Dr. HAY and Dr. FLETCHER. Unfortunately, I did not in many instances ask for a brief account of the disease with which the diarrhoea was associated; but in no case was it the result of typhoid or of the action of drugs, and only one patient had distinct symptoms of enteritis. Often the diarrhoea was slight, the stools being semi-solid.

The results of the examination of seventeen samples of evacuations from different individuals are classified below for the sake of brevity. In cases 1 to 11 the dilution represents the minimum dilution in which the spores were absent; in the remainder the maximum dilution in which the spores were present. The whey of typical milk cultures only was inoculated subcutaneously into guinea-pigs in the proportion of 1 c.c. to every 200 grms. of guinea-pig. If the animal died in about thirty hours or less, the virulence of the culture is described as 'normal;' if death occurred in two to four days it is diminished. If the animal recovered with sloughing of the skin at

† *Medical Officer's Report*, Local Government Board, 1896-97, p. 260.

the site of inoculation, the term pathogenic is used to distinguish from the transient swelling resulting from inoculation with the non-pathogenic bacillus of BOTKIN.

In all, except possibly two or three of the earlier experiments, the milk inoculated had been recently sterilised by not more, usually much less, than five days previously ; a necessary precaution, because stale milk becomes again charged with air (oxygen) by absorption, and is consequently a bad anaerobic medium.

Cases	Enteritidis Spores	Dilution	Nature of Stool	Result of Inoculation
1 to 9	Absent	260	...	...
10	Absent	4	...	...
11	Absent	2	...	...
12	Present	1300	Fluid	Diminished virulence
13	Present	1300	Solid	Pathogenic
14	Botkin ?	1300	Semi-solid	Non-pathogenic
15	Present	260	Semi-solid	Diminished virulence
16	Present	1300	Fluid	Normal virulence
17	Present	65	Fluid	Pathogenic

Cases 1 to 11 were suffering from the following diseases amongst others—rheumatism, morbus cordis, phthisis, acute bronchitis, and pneumonia.

Cases 14 and 15 had cirrhosis of liver, and 17 enteritis.

In cases 1 to 11 enteritidis spores were only proved to be absent in very small portions of stool, while they were occasionally present in still smaller portions ; when the number per gramme must have been enormous. If comparatively large quantities of stool, say from one to three loopfuls, had been tested, it is highly probable the spores would have been isolated in almost every instance.

Having therefore, contrary to expectation, found enteritidis spores in the diarrhoeal evacuations of patients, only one of whom had symptoms of intestinal irritation, several normal evacuations were next examined, but the amount tested was larger than the first series. Samples 1 to 5 were obtained from hospital patients whose bowels were moved about once a day ; number 6 was a healthy individual.

Cases	Enteritidis Spores	Dilution	Nature of Stool	Result of Inoculation
1	Present	150	Relaxed	Diminished virulence
2	Botkin ?	2	Relaxed	Non-pathogenic
3	Present	150	Normal	Normal virulence
4	Present	Small particle	Normal	Diminished virulence
5	Present	Small particle	Normal	Normal virulence
6	Present	2	Normal	Pathogenic

Thus the spores were present in five out of six samples of relaxed or normal stools obtained from different individuals, while they were absent in a large percentage of the diarrhoeal cases, because, I believe, a relatively smaller quantity was tested.

But assuming that the same number of enteritidis spores were present in an equal quantity of faecal material from two persons, one only suffering from diarrhoea, it is incorrect to infer that, therefore, enteritidis is sporing with equal rapidity, and that the spores are equally numerous in the intestines of each ; because the total quantity of diarrhoeal stool passed in a given time will, in most instances, greatly exceed the total quantity of normal stools.

The former is diluted with fluid, and consequently its bulk is increased, so the organisms present are also diluted. In other words, one loopful of a diarrhoeal evacuation represents a much smaller portion of the whole quantity passed in twenty-four hours than one loopful of the normal evacuation. In order, therefore, to compare accurately the number of enteritidis spores present in the intestines of two persons passing normal and diarrhoeal motions, respectively, it would be necessary to collect and measure the total quantity passed in twenty-four hours, minus urine ; then to ascertain the smallest measured portion in which the spores were present, and from that to calculate the total number of spores passed per diem : a tedious proceeding not devoid of practical difficulties.

Now the total quantity of diarrhoeal stool passed in twenty-four hours exceeds that of the normal, partly because it is diluted with water which would tend to lessen the specific gravity. I therefore thought that if diarrhoeal and normal evacuations were made into an emulsion with sterile water and diluted to a standard specific gravity, the bulk of the former would be slightly increased and the bulk of the latter greatly increased ; and then if the same quantity of both emulsions were examined for the spores of enteritidis, a rough comparison of the total number in both kinds of stool might be obtained without collecting and measuring the whole quantity passed in a day. But I have found the method unsatisfactory, because the specific gravity of a diarrhoeal evacuation does not always vary with its consistency.

Below are appended the results of the examination of fourteen consecutive samples of faecal material, the first series were from patients suffering from diarrhoea, the remainder from perfectly healthy individuals who were not in hospital. Each sample was made into an emulsion with sterile water and reduced to a specific gravity of 1005. Next, one cubic centimetre of the emulsion having been previously well stirred, was inoculated into a freshly sterilised tube of litmus milk. Some of the emulsion was also diluted 100, 10,000, and 1,000,000 times, and one cubic centimetre of each dilution was also inoculated into milk. The four milk tubes from each sample were then heated, incubated anaerobically, and at the end of thirty-six to forty-eight hours the whey of a typical milk was inoculated into a guinea-pig in the proportion of one cubic centimetre to every 200 grammes.

DIARRHOEA

Cases	Spores in Dilution of				Result of Inoculation
	1	100	10,000	1,000,000	
1	Present	Present	...	...	Pathogenic
2	Present	Present	...	...	Normal virulence
3	Atypical	...	...	...	Not inoculated
4	Botkin (?)	Botkin (?)	...	...	Non-pathogenic
5	Present	Present	...	...	Normal virulence
6	Present	Present	Present	...	Normal virulence
7	Present	...	...	...	Normal virulence

Case 1 had ovarian cyst, 2 pneumonia, 6 tubercular enteritis, and 7 parenchymatous nephritis.

NORMAL

Cases	Spores in Dilution of				Result of Inoculation
	1	100	10,000	1,000,000	
1	Present	...	...	...	Normal virulence
2	Present	Present	...	...	Pathogenic
3	Present	...	...	...	Normal virulence
4	Present	Present	Present	...	Pathogenic
5	Present	Present	...	...	Pathogenic
6	Botkin (?)	Botkin (?)	...	...	Non-pathogenic
7	Present	Present	...	...	Pathogenic

In order to ascertain whether enteritidis spores were constantly present in normal faeces, five consecutive examinations were made at intervals of about a fortnight from the stools of another healthy individual. The quantity tested was a platinum loopful or less.

In all five examinations the milk tube presented the changes characteristic of enteritidis ; three cultures were inoculated.

Examination	Enteritidis Spores	Results of Inoculation
1	Present	Death in about twelve hours
2	Present	Death in three days
3	Present	Not inoculated
4	Present	Death in about thirty hours
5	Present	Not inoculated

To ascertain how long enteritidis spores in faecal material survived, four samples of stool from the same individual were enclosed in sterile Petri dishes and kept for a period varying from one week to three months. Milk tubes inoculated with one loopful of the faecal matter from the centre of each sample and treated in the usual way, presented the changes typical of enteritidis. Two cubic centimetres of whey obtained from the oldest sample were inoculated into a guinea-pig of four hundred grammes. The animal died in twelve hours with characteristic oedema.

It will be noted in these experiments that the virulence of the cultures, although made from recently sterilised milk, was often sub-normal. However, this diminution may be more apparent than real. I have frequently observed, when a few hours after inoculation the large subcutaneous tumour forms, an escape of the enteritidis-containing fluid from the site of the puncture. If the leak is considerable the guinea-pig usually recovers with local sloughing of the skin, and the virulence of the culture, which may have been normal, is apparently diminished. Leaking is especially liable to occur when, as obtained in the majority of my experiments, the skin of the animal was shaved over an area of the size of a two shilling piece, and, consequently, the protection afforded by the hair removed. That accidental diminution of the virulence may take place is proved by the following experiment. On one occasion two guinea-pigs were inoculated at the same time with milk cultures obtained from the same material. One cubic centimetre of an emulsion of diarrhoeal stool, reduced to a specific gravity of 1005, was diluted 100 and 10,000 times, and inoculated into tubes of recently sterilised milk, *a* and *b* respectively. At the end of two days both tubes presented the characteristic changes, and on cover slip examination, using GRAM's stain, were found to contain apparently pure cultures of enteritidis; spores were absent. Two cubic centimetres of whey

from tube *a* were inoculated into a guinea-pig of 415 grammes, and 2·25 cubic centimetres from tube *b* into a guinea-pig of 465 grammes; both animals were previously shaved. Next day the second guinea-pig was dead with the usual oedema; while the enteritidis-containing fluid was freely escaping through the punctured skin of the first, which recovered with slight necrosis. Thus the apparent difference in the virulence of two similar cultures from the same material was almost certainly due to the accidental enlargement of the inoculation puncture in the case of one guinea-pig: an accident more prone to occur when the animal has been shaved.

It has been shewn that enteritidis spores were isolated in ten out of twenty-four samples of diarrhoea evacuations from different patients, one only having symptoms of intestinal irritation. In five the virulence was normal, and in two diminished, while three were pathogenic, but not fatal. The spores were also demonstrated in the normal evacuations of eleven out of thirteen persons; they were of normal virulence in four cases, diminished in two, and pathogenic in five.

Apparently the bacillus of BOTKIN was present in two samples of diarrhoeal and two of normal stools.

Lastly, the normal evacuations of the same healthy individual were examined on ten occasions at intervals of weeks or months, and enteritidis-like cultures were invariably obtained. Four such cultures were tested by inoculation, in three the virulence was normal, and in one diminished.

The spores were isolated in a larger percentage of normal than of diarrhoeal stools, because in the former the quantity tested was relatively larger than in the latter. The virulence was often subnormal, partly because the site of the inoculation was shaved.

Occasionally the milk cultures were 'atypical.' Such were not inoculated, but they are, according to KLEIN, of diminished virulence or non-pathogenic.

Recently KLEIN isolated the spores of enteritidis of normal virulence in one loopful of stool from one case of sudden fatal diarrhoea, and from a sample of diarrhoea associated with pneumonia; also in from one to three loopfuls of six samples of the 'more or less fluid contents of the colon transversum obtained, post-mortem, from six persons' who died from various causes as epilepsy, peritonitis, cerebral abscess, etc. Lastly, in good-sized lumps of faecal matter obtained, post-mortem, from three out of five individuals in whom 'neither the history or the actual state of the bowel and contents of the colon suggested diarrhoea.'*

Dr. KLEIN also tested the evacuations of forty-three persons suffering from typhoid at different periods of the disease and in convalescence, estimating the minimum number of spores present in the diarrhoeal and non-diarrhoeal stages. He found that 'whereas in the phases of enteric fever associated with typical fluid typhoid stools, these spores of bacillus enteritidis are as a rule *numerously present*, in a

* *Medical Officer's Report*, Local Government Board, 1898-99, p. 329.

considerable percentage of instances (about thirty-nine per cent.) of the typhoid cases wherein these stools had become, owing to constipation or convalescence, normally formed and solid, the spores were altogether absent in relatively considerable amounts of faecal matter. And even in those instances of formed stools in which these spores were found to be present they could only be demonstrated by using large amounts of the faecal matters.' *

Do these results indicate some cause or connection between the presence of enteritidis spores and the formation of the typical fluid typhoid stools? If so, it is curious that the virulence of enteritidis cultures obtained from the cases of 'diarrhoea' with 'typical' stools was normal in twenty,†[1] and diminished in three[2], instances; while on the other hand, it was normal in fourteen[3] and diminished in two[4] cultures from 'solid' stools, diarrhoea being absent.

In my opinion the simplest explanation of the numerical increase of enteritidis spores in typhoid diarrhoea is the fact that as a rule all micro-organisms normally inhabiting the intestines tend to multiply exceedingly when the character of the bowel contents is modified by disease. So it appears highly probably that the increase of enteritidis spores in typical stools is a result, not a cause, of the abnormal conditions of the evacuations.

In conclusion, it is proved that the spores of bacillus enteritidis are constantly present, not only in the abnormal evacuations of those suffering from diarrhoea, whether it is acute or chronic, mild or severe, arising *de novo*, or secondary to organic disease, but also in the formed evacuations of healthy individuals; consequently, bacillus enteritidis, frequently of 'normal virulence,' must be regarded as a common inhabitant of the human intestine.

Since writing the above my attention has been drawn to some observations by Dr. HEWLETT at the Jenner Institute of Preventive Medicine. Adopting KLEIN'S method of isolating the spores by heat incubating them anaerobically in recently sterilised milk and testing the cultures by inoculation of guinea-pigs, Dr. HEWLETT sums up his investigations as follows:—'I have isolated the bacillus enteritidis sporogenes from the dejecta in twelve cases of ulcerative colitis, in one case of ordinary diarrhoea, one case of chronic dysentery, and in eleven out of thirteen specimens of normal dejecta from ten healthy individuals, and the conclusion I have formed is that *this* organism is probably an inhabitant of the normal digestive tract, and frequently to be found in the dejecta.'

Dr. LEGGE also examined a number of samples of dejecta from healthy individuals 'by means of anaerobic milk cultures and microscopical examinations therefrom,' and bacillus enteritidis sporogenes 'seem to be constantly present.'‡

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* *Medical Officer's Report*, Local Government Board, 1898-99, p. 312-328.

† *Medical Officer's Report*, Local Government Board, 1898-99, p. 312-328:

1 Cases 1, 3, 6, 7, 9, 11, 14, 19, 20, 24, 25, 28, 31, 32, 35, 40.

2 Cases 7, 22, 33.

3 Cases 4, 5, 6, 8, 14, 15, 16, 21. 37, 43, 44.

4 Cases 3, 11.

‡ *Transactions of the Jenner Institute of Preventive Medicine*, second series, p. 73.

The second main reason for thinking the epidemic of 1895 was probably due to bacillus enteritidis, was the discovery of the virulent spores of this supposed 'uncommon'* organism in a particular sample of milk. Obviously, it is essential to ascertain the distribution of the bacillus, whether it is common or uncommon, and whether its spores are frequently or rarely present in food.

In the year 1898, KLEIN points out that among the sources of bacillus enteritidis sporogenes are the evacuations of cases of diarrhoea, sewage water, soil and dust polluted sewage, as also horses' dung. But normal evacuations must also be regarded as a source. Again, Dr. BALFOUR STEWART in Liverpool and Dr. HEWLETT in London have isolated enteritidis spores from laboratory dust. They are also present in polluted water, *e.g.*, the River Severn, but never in water supplied to the city of Liverpool. Dr. BALFOUR STEWART has recently drawn attention to the wide distribution of enteritidis sporogenes 'in quite innocent substances,'† for he found the spores from various samples of wheat, barley, oats, oatmeal, flour, rice, cornflour, etc., also from clover and various grasses. In all, 'sixty samples were examined, and forty-one gave an enteritidis-like growth in milk; of these, thirty were fatal to guinea-pigs when inoculated, and eleven were pathogenic, but not fatal.'

It appeared highly probable that enteritidis spores are also present in the atmosphere, I therefore examined the air outside the laboratory by aspirating a measured quantity through sterile plugs of cotton wool; these plugs were then transferred bodily into milk, heated and incubated anaerobically as usual. The volume of air analysed on the four occasions was 480, 2,500, 142, 960 litres. On cultivation each milk tube presented an enteritidis-like growth, the first and third cultures only were tested by inoculation and proved to be of normal virulence. Again, recently-sterilised milk exposed to the open air in sterile Petri dishes for a few hours frequently presents, after heating to 80° C. and anaerobic cultivation, changes characteristic of enteritidis. On one occasion five Petri dishes of sterile milk were exposed in the rooms of a private house for fourteen hours. After incubation three yielded a typical enteritidis-like growth, and contained the usual bacilli. Two of the cultures were inoculated, one was of normal virulence, the other pathogenic. Since, therefore, virulent enteritidis spores constantly occur in the atmosphere and in deposits of dust, the fact that they have been isolated from the surface of wheat, rice, &c., is not surprising.

As the spores of enteritidis are very resistant to heat, and 'have been found to withstand perfectly unharmed a temperature of 100° C. for several minutes, and one of 98° C. for a considerable time,'‡ it is not improbable that in some cooked dishes prepared from flour, oatmeal, &c., the spores survive. For example, STEWART

* *Medical Officer's Report*, Local Government Board, 1895-96, p. 204.
† *Thompson Yates Laboratories Report*, vol. iii, p. 31.
‡ *Medical Officers's Report*, Local Government Board, 1898-99, p. 336.

demonstrated virulent enteritidis spores in two out of two samples of rice, and ANDREWES showed that 'the temperature of a rice pudding removed hot and bubbling from the oven' averaged from '90° C. to 92° C., and nowhere exceeded 98° C.'

But do the spores of this organism ever occur in food which is eaten without cooking? I thought they might be present in such food as strawberries, which, growing near the ground, are liable to be contaminated with particles of manure and soil. Samples of ripe strawberries were therefore brought to the laboratory in paper bags, and their surface sponged with small pieces of sterile wool soaked in sterile milk, the wool was then dropped into tubes of recently sterilised litmus milk and treated as usual.

In twenty-five strawberries examined thus, obtained on three occasions from three different shops and representing about six varieties, the results of milk cultivation were the following :—

Seven tubes contained a typical and fifteen an atypical enteritidis-like growth. The whey from five of the typical tubes was inoculated into guinea-pigs in the usual proportions : two were found to be non-pathogenic, and three pathogenic but not fatal. On routine microscopical examination of the milk cultures, it was noticed that the cover-slip preparations differed from those usually obtained from milk cultures of enteritidis from evacuations, inasmuch, as in addition to the usual bacilli other organisms were constantly present ; the atypical cultures contained many spores.

Of six milk cultures made from the surface of six ripe-red gooseberries, two were typical and four atypical. One typical culture only was inoculated, and proved to be of normal virulence.

Crystallised white sugar was next examined. Six samples of apparently clean sugar were bought in the ordinary way ; and six samples, somewhat dusty through exposure in various shop windows, were supplied in sterile test tubes. A two per cent. solution of each sample was made with sterile milk and treated as usual.

The result of analysis and the weight of sugar tested are given below :—

DUSTY SUGAR

No.	Weight in Grammes	Culture	Result of Inoculation
1	1	Typical	Not inoculated
2	1	Typical	Not inoculated
3	·5	Typical	Pathogenic
4	·5	Typical	Diminished virulence
5	·5	Typical	Pathogenic
6	·5	Typical	Not inoculated

CLEAN SUGAR

No.	Weight in Grammes	Culture	Result of Inoculation
1	1	Atypical	Not inoculated
2	1	Negative	—
3	1	Negative	—
4	1	Typical	Normal virulence
5	1	Atypical	Not inoculated
6	1	Negative	—

Every typical culture contained the characteristic bacilli.

Enteritidis spores are not infrequently isolated from oysters and other shellfish, which are either eaten raw or after cooking for so short a time that probably the spores survive.

I have constantly found enteritidis spores in samples of a large sausage or saveloy of doubtful antecedents, which is made, cooked, and extensively sold by the pork butchers of this city. The saveloy is not recooked after purchase, but should be eaten while fresh, which is rather fortunate, since I obtained, on making agar plates of two samples, from two thousand to three thousand colonies of various organisms per gramme of sausage. When testing for enteritidis spores two or three pieces about the size of a pea were taken from different parts of the interior of the saveloy. The number of typical and atypical milk cultures obtained from eight samples bought from different shops are set down below. Typical cultures only were inoculated.

No.	Typical	Atypical	Result of Inoculations
1	1	1	Normal virulence
2	1	2	Pathogenic
3	2	1	Pathogenic
4	2	1	Not inoculated
5	1	2	Not inoculated
6	2	1	Normal virulence
7	1	2	Not inoculated
8	1	2	Non-pathogenic

It is interesting to note that samples 1 and 2 were subsequently eaten by the laboratory attendants without ill effect.

The pork butchers unfortunately decline to give any information concerning the genesis of the saveloy. Probably, however, they are made from the intestines of the pig, and from odd scraps of meat which have been exposed to dust and dirt, and consequently the presence of enteritidis spores in them is not extraordinary.

Since the epidemic, 1895, the spores of bacillus enteritidis have been frequently discovered in milk. KLEIN isolated them from eight out of ten samples of milk, bought in 'various quarters' of London,* and HEWLETT from eight out of fifteen samples.† ANDREWES examined the milk supplied to St. Bartholomew's on six consecutive days, with positive results on five occasions.‡ Samples of milk sold in Liverpool have been systematically examined in this laboratory during the last fourteen months. About 10 c.c. of each sample were placed in sterile test tube, heated to 80° C., for a quarter of an hour and incubated anaerobically. At first the resulting enteritidis-like cultures were tested upon guinea-pigs. Out of twenty-one inoculations the virulence was normal in nine instances, and diminished in five, four were pathogenic but not fatal, and two were non-pathogenic. Thus the results of inoculation proved that enteritidis bacilli were present in eighty-six per cent. of the enteritidis-like cultures, and forty-two per cent. were of normal virulence.

* *Medical Officer's Report*, Local Government Board, 1897-98, p. 236.
† *Transactions of the Jenner Institute of Preventive Medicine*, second series, p. 75.
‡ *Lancet*, January 1899, p. 9.

The following table gives the number of samples examined every month, and the percentage of enteritidis-like cultures; about half the samples were 'Railway' and half 'Town' milks :—

	Number Examined	Percentage of Enteritidis-like Cultures
November, 1899 ...	23	13
December „ ...	42	30
January, 1900 ...	44	1
February „ ...	50	8
March „ ...	54	27
April „ ...	37	35
May „ ...	50	22
June „ ...	48	10
July „	41	14
August „ ...	47	11
September „ ...	43	9
October „ ...	55	7
November „ ...	35	5
December „ ...	39	5

It appears from these figures, probably owing to the more energetic Public Health Department, that the enteritidis spores are less frequently present in Liverpool than in London milk. I am unable to account for the high percentage of enteritidis spores during November and December, 1899. Although the available figures are not yet sufficient to establish a periodic cycle of bacillus enteritidis sporogenes, nevertheless they appear to indicate that these spores were more common in Liverpool milk during March, April, and May, 1900, when the death rate from epidemic diarrhoea was low, than in the summer months, when the death rate from epidemic diarrhoea was high. As STEWART pointed out, the existence of enteritidis spores in milk is not necessarily an indication of sewage pollution, because in Liverpool milk, at any rate, there is no relation between the presence of bacillus enteritidis sporogenes and of bacillus coli. I think, in many cases, the organism is simply an index of dust contamination.

Believing enteritidis sporogenes is non-pathogenic to the human alimentary canal, Dr STEWART suggested the following experiment.

A litre of new milk was heated for a quarter of an hour at 80° C., and incubated at 37° C. After twenty-four hours, it presented the changes typical of enteritidis. We both ate about three teaspoonfuls of the curd, which was crowded with the characteristic bacilli, and afterwards inoculated a large guinea-pig with 2 c.c. of the whey. Next day, the animal was dead with extensive oedema. The observers continued in normal health. I have repeatedly eaten the greater part of enteritidis-like milk cultures derived from food materials, which subsequently proved to be virulent in guinea-pigs, without evil results.

These experiments are not convincing, but only suggestive ; firstly, because enteritidis does not spore in fresh virulent milk cultures, and consequently the bacilli may have been killed by the gastric juice ; secondly, because healthy individuals are usually able to resist infection by micro-organisms.

The litre of new milk mentioned above, although *not* incubated anaerobically, produced a typical enteritidis culture of normal virulence. In this laboratory, typical enteritidis-like cultures have often been obtained in recently sterilised milk *without* anaerobic incubation in a BUCHNER's cylinder ; not because anaerobiosis is unnecessary, but because the process of sterilisation converts the milk into an anaerobic medium by driving off all or the greater part of the oxygen it has previously absorbed from exposure to the atmosphere. KLEIN has demonstrated that 'stale' milk, *i.e.*, milk which has not been recently sterilised is a bad anaerobic medium because it has become re-charged with oxygen ; and the oxygen may be driven off by re-sterilisation. If two tubes of recently sterilised litmus milk are simultaneously inoculated with enteritidis sporogenes, heated for one quarter of an hour at 80° C. and incubated at 37° C., the one in a BUCHNER's cylinder with pyrogallic acid and potash, the other aerobically, it will be found that the first sign of the growth of the bacilli is bleaching of the litmus in the lower part of *each tube*. But whereas the culture in the BUCHNER's cylinder will soon be totally bleached, the other becomes red at the top, and eventually when the formation of gas and whey is complete, the redness will extend in two or three days to the curd at the bottom. If now the anaerobic culture be removed from the BUCHNER's cylinder, its curd though bleached will also become slowly reddened by the gradual absorbtion of oxygen. That is to say, in most, if not all, instances the only apparent difference between two enteritidis cultures in recently sterilised litmus milk, incubated anaerobically and aerobically, respectively, is reddening of the litmus at the top of the aerobic culture. It would probably be found that both cultures were equally virulent to guinea-pigs.

I have found that if a stream of sterile air is passed, for a few minutes, through a milk culture after inoculation with enteritidis sporogenes, and heating for quarter of an hour at 80° C., the characteristic change takes place several hours later

than in a control culture, though it is usually still typical. If the milk was completely oxygenated by this process it is probable that an atypical culture would result.

To return, I think it has been shown that bacillus enteritidis sporogenes is, as HEWLETT thought, a ubiquitous organism ; that enteritidis spores of normal virulence contaminate food which is eaten with impunity. The wide distribution of the bacillus was unknown when it was first called a 'diarrhoeal'* organism. I admit it is possible that under certain unknown conditions bacillus enteritidis sporogenes may be pathogenic on the human intestine ; but so may any other bacillus. Referring to the frequency of enteritidis spores in milk consumed without toxic effect, KLEIN suggests that 'much may depend on the strain of bacillus enteritidis which obtains entrance into the milk,' also on certain atmospheric 'chemical or other' conditions.

The diarrhoea epidemic at St. Bartholomews in 1895 'appeared referable' to the presence of enteritidis spores in certain milk. But there is no definite proof that the spores *were* present in this toxic milk, for no specimen of it was available for examination. It is true that enteritidis spores were detected in a sample obtained three days after the epidemic from one farm, which had supplied the hospital on the day of the outbreak with only about nine per cent. of the total milk consumed.

The second epidemic was also attributed to milk, which unfortunately does not appear to have been submitted to any chemical or bacteriological examination beyond the isolation from it of a particular 'ubiquitous' microbe.

Rice pudding was the incriminated diet in the third epidemic. Dr. ANDREWES states that, on microscopical examination of the pudding, not only was an enteritidis-like bacillus detected, but also other organisms, namely, 'cocci and small bacilli resembling lactic acid bacillus.' Bacillus enteritidis was isolated in a non-sporing condition from the pudding ; yet no further investigation was made into the 'nature' of the other organisms.†

But, even if the enteritidis in the rice pudding was 'the direct cause of the disease,' from what ingredient was it derived, from the rice, from the sugar, or from the milk ? And how can sanitary authorities hope to prevent epidemics of diarrhoea due to a bacillus which, though frequently present in 'quite innocent substances' and eaten with impunity, is occasionally exceedingly pathogenic ?

The third main reason for associating enteritidis with diarrhoea appears to be its pathogenicity to rodents as guinea-pigs. But even if cultures of a micro-organism grown in favourable conditions are pathogenic when inoculated under the skin of a guinea-pig, why shonld that organism necessarily, or even probably, be pathogenic to the intestine of man ? Guinea-pigs fed on cultures of enteritidis are not attacked with diarrhoea, which is truly fortunate considering the bran and oatmeal they eat frequently contain enteritidis spores of normal virulence.

* *Medical Officer's Report*, Local Government Board, 1895-96, p. 204.
† *Lancet*, January 1899, p. 9.

To sum up, the bacillus enteritidis was first discovered in connection with a specific epidemic of diarrhoea. It was thought that this bacillus probably caused the outbreak, because it was assumed to be an *uncommon* organism, and was present in the evacuations of the persons attacked and also in a sample of milk supposed to be similar to the original toxic milk. The truth of this supposition was confirmed by the occurrence at a later date of two similar epidemics, apparently also due to the same bacillus.

Meanwhile it was discovered that the organism in question has a very wide distribution, is commonly present in the air and in dust, and is frequently consumed in food *without* producing diarrhoea.

Again, it has been proved that enteritidis occurs in the evacuations from chronic diarrhoea, from diarrhoea secondary to other diseases, and from healthy individuals; it is in fact a normal inhabitant of the human intestines, and consequently its presence in the evacuations obtained in certain epidemics has *no special significance.*

But, as KLEIN pointed out, the bacillus of Gaertner, bacillus proteus vulgaris and streptococcus pyogenes, although also normal inhabitants of the intestine, may under certain unknown conditions set up violent diarrhoea.* The value of this analogy is considerably lessened, because it is highly probable, if not actually demonstrated, that cultures of these organisms are much more virulent to animals when producing diarrhoea than when they are quiescent; whereas, the spores of enteritidis, obtained from normal or diarrhoeal evacuations, are equally virulent.

I believe it is impossible to demonstrate the causal relationship between bacillus enteritidis and epidemic diarrhoea by isolating it from the evacuations and from food, or by testing its virulence upon guinea pigs.

KLEIN, however, records that the swollen lymphoid follicles in the intestine of a fatal case of cholera nostras contained, on microscopical examination, in almost pure culture, an organism 'morphologically like' bacillus enteritidis.†

So it is important to ascertain whether enteritidis sporogenes is constantly present in the inflamed intestinal walls of those who have presumably died from its activity, making sure that its presence is not the result of post-mortem invasion.

Again, does the serum of patients suffering from epidemic diarrhoea exert any agglutinating action upon enteritidis bacilli?

In conclusion, I submit, there is at present no satisfactory evidence that bacillus enteritidis sporogenes is a cause of acute, or of epidemic diarrhoea.

* *Medical Officer's Report*, Local Government Board, 1898-99. p. 332, 336.
† *Medical Officer's Report*, Local Government Board, 1897-98, p. 283.

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FURTHER NOTE ON BILE SALT LACTOSE AGAR

By ALFRED MacCONKEY

In a previous article of this Volume (p. 40) of the *Thompson Yates Laboratories Reports*, I gave an account of some rough experiments made with this medium. These experiments have been continued, and GRÜBLER's pure salts have been used instead of the impure commercial salts. Cholalic acid, glycocholic acid, taurocholic acid, sodium glycocholate and sodium taurocholate have been experimented with, and it has been found that it is only the sodium taurocholate which gives the characteristic reaction, namely, a cloudiness or haze in the medium in the vicinity of the colonies. This salt also seems to exert a more pronounced inhibitory effect than the glycocholate. The medium was made in the following way :—

1,000 c.c. of tap water were put into a flask, and 2 per cent. peptone, and 0.5 per cent. of sodium taurocholate, and 1.5 per cent. of agar added. The flask was then put into the autoclave and kept at 105° to 110° C. for about one-and-a-half hours. The mixture was cleared with white of egg, using as little as possible, and filtered. Then 1 per cent. of lactose was added to the filtrate, the medium was distributed into test tubes, ten c.c. in each, and sterilisation effected by fifteen to twenty minutes in KOCH's steriliser on each of three successive days.* The plates made were incubated at 42° C. for forty-eight hours. Organisms which produce acid with lactose in forty-eight hours give a characteristic reaction in this medium. The acid causes a precipitation of the taurocholate with the formation of a cloudiness round the colony. Thus there is a marked difference between the colonies of such organisms as the B. typhi abdominalis and those of the B. coli communis group.

B. Typhi Abdominalis.

Surface colonies—Small, round, raised, and semi-transparent.

Deep colonies—Lens-shaped, white, and opaque.

The medium remains quite clear.

B. Coli Communis.

Surface colonies—Roundish or irregular in shape with flattened top, opaque-white with a small spot of yellow or orange in

* If the medium be overheated, changes take place in the lactose and the B. typhus will give a haze.

the centre. A few have a haze round them. The size is about twice that of B. typhi abdominalis.

Deep colonies—Lens-shaped, orange white, and all have a haze round them.

This haze makes the colonies appear larger than they really are. A drop or two of a solution of ammonia placed upon the colony will cause the haze to disappear. If the plates be exposed to the light for several days it will be found that the colour of the colonies has deepened and that those on the surface have a tendency to become mucoid.

B. Enteritidis (GÆRTNER) *B. of Hog Cholera* (FLEXNER) *B. of Psittacosis* (NOCARD)	Colonies similar to those of B. typhi abdominalis
B. Pyogenes foetidus *B. Neapolitanus*	Colonies similar to those of B. coli communis.
B. fluorescens liquefaciens	Slightly raised, flattened, roundish, translucent, greenish-white colonies with very slight fluorescence.

Cocci.

Surface colonies—Round, raised, opaque-white.

Deep colonies—Usually opaque white. Under the microscope they appear black with a rough irregular hairy edge.

The following few organisms have been tried and apparently do not grow on this medium at 42° C.

B. Megatherium.
B. Subtilis.
B. Filamentosus.
Sarcina lutea.
Torula alba.

The following experiment was performed to ascertain the inhibitory power at 42° C. of this medium upon ordinary earth organisms.

About two to three grammes of garden mould were shaken up with 100 c.c. of tap water and 1 c.c. of the mixture put into a tube and poured into a plate.

At the end of forty-eight hours there were, besides many moulds, ten colonies (both surface and deep). All these colonies were subcultured, and nine gave a growth. These nine were then cultured in different media and the reaction noted. The incubation temperature was always 42° C.

The following table gives the reactions of these nine organisms :

		Broth	Litmus Milk	Litmus Glucose Broth	Litmus Lactose Broth	Agar Stab.	Gelatine Stab.	Potato	Indol	Gram's Method	Tauro-cholate Agar Stab
1	Sluggishly-motile short bacillus	General turbidity	Acid : no clotting	Acid and gas	Acid : no gas	Moist, grey-ish-white growth	Filmy, opa-que white	Almost in-visible moist growth	(?)	—	Haze
2	„ „ „	„	„	„	„	„	„	„	„	—	„
3	Motile short bacillus	„	Acid and clotting	„	Acid and Gas	„	„	Moist whit-ish growth	+	—	Haze and Gas
4	„ „ „	„	„	„	„	„	„	„	+	—	„
5	„ „ „	„	„	„	„	„	„	„	+	—	„
6	„ „ „	„	„	„	„	„	...	„	+	—	„
7	Coccus in short chains ...	Very slight general turbidity	„	Acid	Acid	Grey-white growth	Liquefied	Invisible	—	+	No haze
8	„ „ „ ...	„	Alkaline	„	No change	Opaque and white	Opaque-white, scan-ty growth	„	—	+	„
9	„ „ „ ...	„	Acid	„	„	„	„	„	—	+	„

On gelatine plates there was no difference to be made out between the colonies of the various bacilli. They were all irregular-shaped, filmy, and translucent. Some were more heaped up and opaque in the centre, but that was all.

It is noteworthy that all the bacilli appear to belong to the B. coli communis group.

The glucose broth and lactose broth used were two per cent. solutions of peptone in water, to which 0·5 per cent. glucose and 1 per cent. lactose had been added respectively. Into each tube was put one of Durham's fermentation tubes.

As an ordinary nutrient agar plate made from the same earth was over-crowded, the inhibiting effect of the taurocholate medium is most marked.

It may, therefore, be recommendcd to bacteriologists as an addition to the means at their disposal for the isolation of the B. typhosus.

NOTE ON THE STAINING OF FLAGELLA

By ALFRED MacCONKEY

It is a generally accepted idea that staining the flagella of bacteria is difficult to accomplish, and that a 'mordant' is necessary to fix the dye in the substance of the flagellum. Is this idea correct? A 'mordant' is defined as 'a substance used to fix colours in stuffs.' Is this what takes place in the process of staining flagella? At first sight this would appear to be the case, for we find that flagella will not usually stain unless a 'mordant' be used. A little consideration, however, raises doubts as to the correctness of this interpretation of the process. If the 'mordant' acts merely as a fixing agent it should not leave much effect upon the size of the cilia, which should, within limits, appear always of the same thickness; and the depth of colour should depend upon the degree of concentration of the 'mordant' and stain, upon their inter-reaction, and upon the length of time the film was subjected to their action. But, in practice, we find that this is not quite the case. The thickness of the flagella depends upon the strength of the 'mordant' and upon the length of time it is allowed to act; and the depth of colour depends upon the thickness of the substance to be stained. If the flagella are very fine, the strength of the staining fluid and the duration of its application make very little difference, for the flagella will always appear faintly stained; whereas, if the flagella be thick, a comparatively brief application of the stain will dye them a deep colour.

These facts suggest two explanations of the action of 'mordants.' In the first place, the action may be due to the deposition of a precipitate upon the outside of the flagellum, this precipitate being dyed in the subsequent staining process.

In the second place, the 'mordant' may cause the flagellum to swell and become thicker. Thus it would produce a greater depth and breadth of substance for the stain to act upon.

We know that, to be visible, a line must have a certain breadth, and it is quite conceivable, considering the size of bacteria, that their flagella may be too fine to be seen even when stained. Of these two explanations, I am inclined to favour the second for the following reasons:—

1 In all the usual methods, the flagella when stained appear to be thicker than we suppose them to be in reality, and the organisms themselves are larger than when stained in the ordinary way.

2 Certain mixtures, which, when freshly made, cause the flagella to be thick and to stain deeply, after a time lose their power. This power is not lost all at once, but only gradually, and this declination is associated with an increasing fineness of the flagella and decreasing depth of colour. They become finer and finer, and more faintly stained, until finally they are no longer visible. At the same time the bodies of the organisms also become smaller, though they stain equally well. By none of the methods I have used have I succeeded in obtaining very fine deeply-stained flagella. When fine, they have always been faintly coloured and when thick more deeply dyed.

3 If a 'mordant' acted merely as a fixing agent, subsequent treatment with acids should have some effect. After using the 'mordant,' I have kept films in contact with Acid. Sulph. twenty-five per cent., Acid. Acet. ten per cent., and Acid. Nitric. ten per cent. for several minutes without any effect upon the subsequent staining of the flagella.

If, however, spirit be used instead of the acids, the flagella can only be stained faintly, or are not visible. This later result might be used in support of the 'precipitate' theory, the precipitate being soluble in spirit. But it does not invalidate the theory of 'thickening,' as the spirit may cause shrinkage by dehydration. The method used in these experiments was the following :

(*a*) De Rossi's potash tannic acid. Tannic acid twenty-five per cent., dissolved by heat in one per cent. solution of caustic potash in water. This is saturated with fuchsin.

(*b*) Perchloride of iron ten per cent. dissolved in water + five per cent. glacial acetic acid.*

(*c*) Water + five per cent. glacial acetic acid.

The films are made in the usual way, allowed to dry in the air, and then exposed to formalin vapour for about half an hour.

Equal parts of (*a*) and (*b*) are mixed in a test-tube, and boiled. Then an equal bulk of (*c*) is added, the whole shaken up and filtered twice. This mixture is allowed to remain in contact with the film for a time, which varies with the organism used and the result required. From two to seven minutes is a good time for B. typhosus.

The cover slip is then well washed in water (treated with acid if preferred, and washed in water), and stained for about ten minutes in a warm solution of one of the ordinary dyes (an alcoholic-aqueous, one to four or weaker solution of gentian violet gives good results), well washed, dried and mounted in balsam.

The 'mordant' retains its properties for a few hours only.

In specimens stained by this process the protoplasm of the organisms is somewhat enlarged. In many cases the protoplasm appears to be surrounded by a 'capsule,' or 'thickened cell membrane,' which may or may not be stained.†

* This solution seems to lose its power after a few days.

† C.f. *Bacteriol. Diagnost.*, Lehmann and Neumann, Vol. II., p. 18.

When this 'swollen membrane' is visible the flagella appear to be prolongations of it and to have no connection with the protoplasm of the body of the organism.

4 I isolated from sewage a spirillum, which, in liquid media, developed a very long terminal flagellum, and this flagellum was plainly visible in every specimen stained in the ordinary way, *i.e.* without the use of 'mordants.' This flagellum must then have been composed of a substance quite different from that of the flagella of other organisms, a substance which absorbed ordinary dyes ; or it must have had the same composition as any other flagellum and have been visible merely because it was thicker. No adequate proof has been brought forward that the flagella of various organisms differ in their composition, and therefore the reasonable presumption is that it was its breadth which rendered this flagellum visible.

Unfortunately, circumstances prevented for a time further experiments with this organism, and it died. I have not had an opportunity of trying the effect of 'mordants' on it.

For these reasons I venture to suggest that the present ideas of flagella staining may be wrong, that flagella may stain with the ordinary dyes, but are invisible on account of their fineness, and that the so-called 'mordants' may act merely by causing the flagella to swell and thus become visible when stained.

operation, and appeared healthy ; there were no secondary growths found. The amount of urine increased after the operation, the average for the seven following days being 14·7 ounces. The total urea passed in twenty-four hours was estimated on thirteen occasions after operation, the average being 128·8 grains.

The charts shew an apyrexic curve, with the exception of three unexplained and unimportant rises. The child increased in weight, and his general condition on leaving hospital was excellent. The dark colouration of the skin became distinctly more noticeable. He was seen on December 28, 1900, when to all appearance he was in robust health.

Pathological Report—The growth weighs thirty-eight ounces. It is oval in shape, but the outline is very irregular owing to the projection of lobes of growth, chiefly on the inner aspect ; one very prominent lobe projects forwards and upwards from the inner and anterior margin. The whole mass is covered by a fibrous capsule ; this is comparatively thinned over the prominent portions of the lobes, but strongly marked in the intervals between them. At the lower and external pole lie the remains of the renal tissue ; the upper border of this is represented by a sharp edge on the outer aspect of the mass, which can be lifted away from the growth with the handle of a knife ; the handle easily separates kidney and growth inwards and downwards for two inches ; below this they appear to be incorporated with one another. The renal tissue forms the outer and lower angle, the inferior border, and the greater part of the inner and lower angle of the tumour. The renal artery is seen between renal tissue and growth on the inner aspect, in front of a line bisecting the mass from the inner side ; it disappears under the edge of the compressed renal tissue, but by separating this from the growth, the artery can be traced between them for about an inch, when it enters the kidney substance ; the direction of the artery is downwards, forwards, outwards, and then backwards to the hilum.

The stump of the severed ureter lies behind the line bisecting the growth from within outwards ; its direction is almost directly downwards and slightly outwards, round the inferior and inner pole of the new growth to end, after about half an inch of course between kidney and growth, in the pelvis. Its termination could not of course be seen before bisecting the mass. This was done from the inner aspect and the two halves separated.

The renal tissue was then seen to be represented by a strip about 5 m.m. in thickness for the most part, but in the lower half of this a column of new growth, spherical in section, bulges into the pelvis, and is bounded on the outside by the remains of cortex, and on the inside by the pelvic lining.

This column forms a valuable clue to the exact anatomical relationship and the place of origin of the growth, as it is in direct continuity with the main mass. The pelvis on section is crescent shaped, the concavity of the crescent being occupied by the inferior border of the growth, and no renal tissue is distinguishable here outside the

pelvic lining. The convex border of the crescent is formed by the above-mentioned strip of kidney tissue, except where the spherical column of new growth bulges into the pelvis.

The pelvic walls are in contact, but the extent of the lining points to there having been some dilatation. The ureter is patent.

The section of the growth shews a division into irregular lobules, but there are also septa marking off four lobes, one forming the apical portion of the mass, one the central and much the largest portion, and two smaller on the inferior and outer aspect of the central lobe. The upper lobe can be lifted off the central lobe by a slight blunt dissection on the anterior and outer parts, but posteriorly it is blended with the latter. The lower two small lobes are less perfectly differentiated. The lobes differ in naked eye structure; the central mass appears to consist largely of fat with comparatively scanty interstitial matter; the upper lobe is greyish in colour as contrasted with the yellow of the central lobe, and no fatty tissue is appreciable; the same applies to the most external of the other two lobes, while the fourth resembles the central.

Distributed throughout the growth are many small cysts, the largest about the size of the head of an ordinary tin tack.

The relations of the new growth to the remains of the renal tissue enable one to decide with certainty its seat of origin.

The relation of the growth to the lining of the pelvis, and more especially the position of that out-growth which lies between pelvis and cortex at the lower pole of the growth, shows that it originated in the renal tissue itself.

The position of the artery and the ureter show that it originated above the hilum, and about centrally as regards the antero-posterior dimension. Thirdly, the position of the renal remains enables one to decide that the point of origin was on the inner face of the kidney, the growth, by extension, displacing the kidney downwards and outwards, or rather causing it to rotate through a segment of a circle, of which the hilum is the centre, the direction of rotation being from right to left. The seat of origin was therefore in the substance of the kidney, and in the upper and inner quadrant. As stated above, the upper edge of the renal tissue on the outer aspect of the growth is sharply defined, and it can be dissected off without tearing through anything except fibrous bands. This first suggested the idea that the growth was extra-renal in origin, but the other relations negatived this. Evidently the extension of the growth was first in an upward direction, subsequently, meeting the resistance of the capsule, the pressure would be exerted in an outward and downward direction.

The well-marked lobulation probably has relation to the original lobulation of the kidney. The lobe which forms the apex of the growth is so distinctly marked off, that it was thought to represent the adrenal body. Histologically, however, this was not confirmed.

Histology—There is a certain uniformity throughout the whole tumour in that the various elements of which it is composed are present in all parts, but they are very unequally distributed.

Central Mass—Fatty tissue predominates. Other elements appear as scattered islands and tracts in the sections. The fat is enclosed in a fine connective tissue mesh.

Strands of striped muscle cells run in all directions. The striation of many of the cells is easily seen, but in many also it cannot be distinguished. The latter cells are for the most part uninucleated and tapering, the striated cells are stouter and often shew more than one nucleus. All are evidently young cells not far advanced in development. Strands of young fibrous tissue are also present, but more scantily than the muscle tissue; the cells are narrow, with long tapering nuclei.

These bundles of muscle and fibrous tissue can all be traced to connections with the cells of what I will for the present call the *Pseudo-cystic formations.*

The larger of these bodies are easily seen macroscopically as crateriform depressions on section; other forms of them are represented by white dots.

The histology of these bodies is as follows:—

The smallest and youngest consist of—

1 A limiting layer of flattened cells, of connective tissue type.

2 A mass of polyhedral cells within, with large nuclei staining deeply.

From this simple structure they can be traced through a series of changes partly degenerative, partly proliferative.

In the first stage of these changes the cells centrally placed develop a squamous cell body, oval or irregular in shape, and the nuclei stain less distinctly. The cell bodies are hyaline, and between them branches a delicate reticulum (Fig. I).

It is convenient to follow these central cells in the first place, and then to consider those peripherally placed.

The squamous cells next become somewhat shrunken, the nuclear outlines are blurred and the intercellular reticulum is more distinct. The cellular layers then split up into ribbons and separate from one another, and the reticulum consists of strong fibrils circularly arranged (Fig II).

Lastly, the central parts are represented by a (Fig III) whorl-like arrangement of flaky fibrils, the cells having disappeared entirely. In some specimens even this has disappeared, and the structure is a true cyst, containing occasionally some granular debris.

These changes are of course degenerative. The evolution of the peripheral cells is, however, in the direction of proliferation. The limiting layer is usually quite indistinctly marked off from the cells immediately within; these are in many specimens set endwise on the outer layer and are cubical in shape, but this arrangement is not constant. From these peripheral cells to a greater or less degree in all specimens, tracts and columns of cells originate and pass out in all directions into the fatty matrix.

These cells are round cells of sarcomatous type. Where they take origin

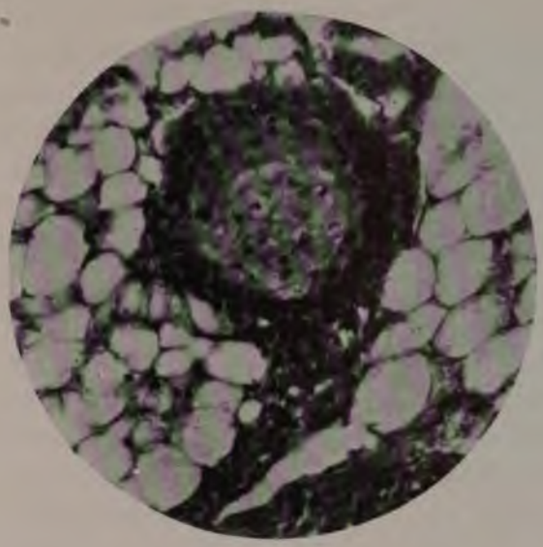


FIG 1

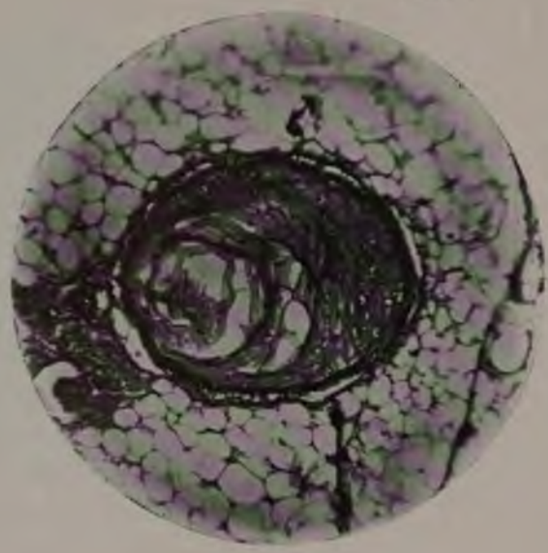


FIG. 2

FIG 3

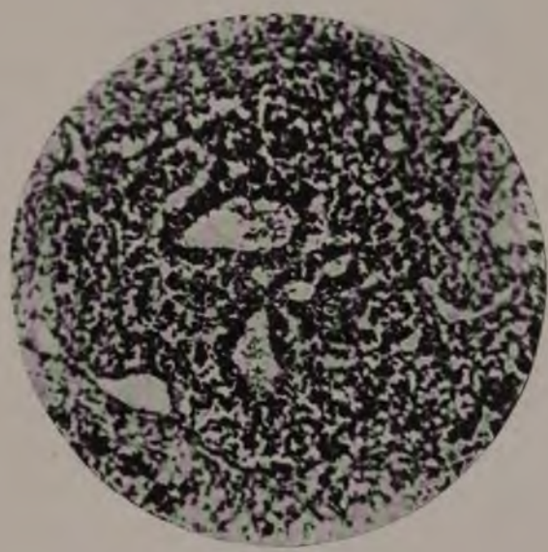


FIG. 4

FIG 1—Shews a pseudo-cyst (Malpighian Corpuscle) in early stage. Central cells squamous or breaking up. Nuclei stain faintly. Peripheral cells proliferating. Edge of sarcomatous mass below.

FIG. 2—Shews pseudo-cyst later. Centrally squamous cells breaking up into ribbons. Fibrillation distinct. To the left a sarcomatous out-growth from the periphery.

FIG. 3—Shews complete fibrillation of interior of pseudo-cyst. Sarcomatous tissue below.

FIG. 4—Shews tubules with broken contour, from the cells of which originate, by proliferation, a mass of round or irregular cells ; the alveolar arrangement not being re-produced.

from the peripheral cells of the pseudo-cysts the outlines of the latter are interrupted (Figs I and II); specimens which are embedded in sarcomatous structure shew no definition except where the central squamous cell passes into the polyhedral type, the latter passing insensibly into the somewhat smaller and rounder sarcoma cell. In some specimens, however, even thus embedded there is a single row of cubical cells with large nuclei outside the squamous cells.

The peripheral cells of the pseudo-cystic bodies, therefore, give origin to sarcomatous elements. But the sarcomatous elements in this main central lobe which we are now considering are not exclusively derived from the pseudo-cysts. Adjacent to many of these, *Tubules* lined by cubical cells are seen, cut in various directions. Their outlines are interrupted by proliferative protrusions of these cubical cells, which, growing outwards, become sarcomatous in type. Scattered tubules, the cells of which are proliferating in this way, are also seen away from the proximity of the pseudo-cysts.

The muscle and fibrous tissue cells forming the strands, are in connection with the cells of the limiting layer of the pseudo-cysts for the most part.

Apical Lobe—This portion of the growth shews microscopically a much smaller proportion of fatty tissue than the central mass. Pseudo-cysts are seen here also, but none are degenerated to the stage of complete fibrillation, but consist centrally of large squamous cells with a fine reticulum. Sarcomatous tissue forms the main mass of this lobe, developed as before from the outer layers of the pseudo-cysts. However, tubules are more numerous here, and most of the sarcoma cells originate from the cells of these (Fig. IV). In fact the outlines of these tubules are usually quite irregular and broken. Muscle and connective tissue cells are distributed throughout the sarcomatous tissue.

Sections from the inferior lobes of the growth shew an almost uniform sarcomatous structure, with some interstitial fatty tissue. Throughout the structure the outlines of scattered and irregular tubules are seen. None of the pseudo-cystic formations were seen in the sections from this portion of the growth.

The vascularity of the different lobes varies. Where the sarcomatous cells are most numerous the vessels are many and large; on the other hand, where fatty tissue predominates they are small and very few in number.

From this description of the histology of the tumour it will be seen that the whole structure is traceable to the cells of certain tubules, and what have been provisionally termed pseudo-cysts. The significance of these two elements must now be considered. The kidney, with the exception of the collecting tubules, is developed from a portion of the intermediate cell mass, posterior to the Wolffian Body, and the first elements differentiated are the Malpighian Corpuscles and convoluted tubules. These both originate as solid masses of mesoblastic cells, and are subsequently hollowed out, the Corpuscle developing at the blind end (that not in connection with the peritoneal involution). The foetal Malpighian Corpuscle consists of a limiting

layer, and a lining of epithelioid cells within, polyhedral or even cubical. Apart from the capillaries which are present in the developed glomerulus, the glomerular tuft is formed by a local proliferation of these epithelioid cells, and of the *membrana propria* or limiting layer, these both covering and passing in all directions between the capillary recticulum. The epithelioid cells become squamous in the adult state.

This structure is identical with a definite stage in the changes of the pseudo-cystic bodies. It has been seen that a few young specimens appear as solid masses of polyhedral cells, encircled by a limiting layer of flattened cells, and that later the central polyhedral cells become squamous.

The absence of the glomerular capillaries does not negative the identity, as they are a secondary in-growth. In their absence no glomerular cavity would develop, depending as it does on secretion derived from the capillaries, and instead, the squamous cells which normally form a lining and a support for the internal structure of the glomerulus, fill the whole interior of the limiting layer. The pseudo-cysts are therefore to be looked upon as Malpighian corpuscles. The structure is sufficient to decide the identity, and it is supported by the presence of the tubules which lie adjacent to and around the pseudo-cysts. Whether these tubules are in this position or more isolated, they must be considered the representatives of the primitive tubules from which the uriniferous tubules develop.

The new growth is therefore traceable to a perversion of the cellular elements from which, under normal development, Malpighian corpuscles and uriniferous tubules arise. The result of this perversion is the production of a tissue largely consisting of fat cells, but also in varying proportion of round cells, with large nuclei in a scanty stroma ; in addition young striated muscle cells and young connective tissue cells are unequally distributed throughout the structure. A name cannot be given to such a tumour on the general principle that malignant tumours arising from glandular epithelium are carcinomata, and those arising from connective tissue are sarcomata. The principle would seem to apply to growths originating from a substratum of adult cells, but in such a case as the present its application presents, among others, the following difficulties. The cells in many situations have obviously arisen as a proliferation of those of the tubular epithelium, on the other hand the resulting growth is composed of solid masses of round and irregularly shaped cells, and the structure is what is commonly called sarcomatous.

Birch-Hirschfeld* in describing a somewhat similar tumour remarks that these primary malignant growths of the kidney belong to no one group in the present classification, and gives to that under his consideration the name 'sarcomatous glandular tumour of the kidney.' Other authors have remarked on the same difficulty in nomenclature, and until the growths which apparently arise in connection with irregularities in embryological processes can be separately classified, it will be impossible to arrive at any satisfactory solution.

* Birch-Hirschfeld. *Ziegler's Beitr. Z. Path. Anatom.* Bd. xxiv. P. 343.

The late Dr. Myers

In Memoriam

WALTER MYERS, M.A., M.B., B.C. Cantab., B.Sc. London, who, on a mission of the Liverpool School of Tropical Medicine to Para on the Amazon to investigate yellow fever, died of that disease in January, 1901.

'From the service of truth and humanity upon earth, in the morning of his manhood, he passed to GOD.'

On Sunday, the 20th of January, the Tropical School learnt by cablegram from Para that both members of the Yellow Fever Expedition had contracted yellow fever, Dr. MYERS being reported critically ill. On Monday, January 21, the sad news of his death was received.

The following two letters arrived on February 9, from Para.

Letter from His Excellency, the Governor of the State of Para, to the Secretary of the Liverpool School of Tropical Medicine :—

SIR,

It is my painful duty to inform you of the death of Dr. WALTER MYERS, who—a victim to his love of science and to humanity—has just succumbed to the terrible illness which robs us of so many precious lives, whilst himself employed in combating this terrible disease. In the name of the State of Para, and interpreting the feelings of regret with which we are all possessed, I desire hereby to express to the Liverpool School of Tropical Medicine our deep sympathy for the loss they have just suffered. To the honourable family of the deceased, who has left us a ray of light to illumine the field of science and an example of altruism and abegnation, I also desire to offer our very sincere sympathy in their bereavement.—I remain, Sir, yours sincerely.

(*Signed*) DR. JUAN PAES DE CARVALHO.

Letter from Mr. A. J. BEALE, of the firm of Messrs. SINGLEHURST, BROCKLEHURST & Co., Para, to Mr. GEORGE BROCKLEHURST (Member of the Committee of the Liverpool School of Tropical Medicine).

DRS. DURHAM AND MYERS

It is with very great regret we have to inform you of the death of Dr. WALTER MYERS, of the Yellow Fever Commission, which occurred on the 20th instant, at 5 p.m., from yellow fever at the Yellow Fever Hospital. Dr. DURHAM is recovering rapidly, and was yesterday able

to leave his room for a short time. Both doctors were attacked with the fever on the 16th instant, Dr. DURHAM's case declaring itself first, his colleague procuring him a carriage to convey him from the L. S. Institute to the hospital. A few hours later Dr. MYERS was found to be with a high fever, and it was at once found that in his case the disease was of a malignant type. They were treated at the Yellow Fever Hospital, and everything possible was done for them. Besides the nurses, a doctor was always at the hospital, and Dr. PAES DE CARVALHO was with them two or three times daily. It is supposed that both doctors contracted the disease during an autopsy on a very malignant case of yellow fever, which had occurred at the hospital two or three days previous.—Yours truly,

p. pro. SINGLEHURST, BROCKLEHURST & CO.,
(*Signed*) A. J. BEALE.

PARA, BRAZIL, *January* 23, 1901.

The following obituary notice of Dr. WALTER MYERS appeared in the *British Medical Journal* of February 2, 1901.

WALTER MYERS was born twenty-nine years ago, and was the son of Mr. GEORGE MYERS, a well-known Birmingham citizen. He was educated at King Edward's School. After leaving school he studied in the Biological Laboratories of Mason College from 1888 to 1890 while preparing for the Intermediate Examination for the B.Sc. Lond. degree, which he took in 1892. In the Botanical Laboratory of Mason College—where he won the Senior Botanical Prize in 1890—he developed that strong taste for microscopical study which played an important part in subsequently determining his mental bias in his purely medical studies. He proceeded to Cambridge in 1890, having won a Natural Science Scholarship at Caius College, and graduated M.B., B.C. in 1897.

Those who knew MYERS and the work that he has carried on in recent years know how great a loss his death will be to the science of medicine. The late Professor KANTHACK was early struck by the scientific enthusiasm and acumen displayed by MYERS when he came to work in his laboratory, and before the late professor died he recommended MYERS for the John Lucas Walker Scholarship on the understanding that he should continue his work on immunity, more especially with the ferments found in diseased products and in the blood.

After working for a short time at Cambridge, MYERS received word that he would be allowed to continue his investigations in Professor EHRLICH's Laboratory, where he entered so thoroughly into the spirit of the place that he soon produced further excellent work on cobra poisoning, and commenced an investigation on the action of the various forms of proteids and their anti-bodies. This work, of which he had published a preliminary account before he left on the Yellow Fever Expedition, following up that already carried out by EHRLICH, is one of the most important of recent contributions to the study of immunity.

His more important papers: 'Cobra Poison in Relation to Wassermann's New Theory of Immunity' (*Lancet*, 1898, vol. ii, p. 23); 'The Action of Cobra Poison on the Blood: a Contribution to the Study of Passive Immunity' (in collaboration with Mr. J. W. W. STEPHENS, M.A., M.B. Cantab.) (*Journal of Pathology and Bacteriology*, 1898, vol. v, p. 279); 'On the Interaction of Toxin and Antitoxin: Illustrated by the Reaction between Cobra Lysin and its Antitoxin' (*op. cit.*, 1900, vol. vi, p. 415); MYERS (and STEPHENS): 'Influence of Cobra Poison in the Clotting of

Blood and the Action of Calmette's Antivenomous Serum upon the Phenomenon' (*Proceedings Physiological Society*, p. I, in the *Journal of Physiology*, vol. xxiii) ; 'On Immunity against proteids' (*Lancet*, 1900, vol. ii, p. 98) ; indicate the work on which he was engaged, how much he had already done, and what promise he had given for the future.

Only those who knew him well had any idea of the kindliness of his disposition, his thoughtfulness for others, and his enthusiasm for his work. He was a Cambridge man of the best type, with a well-stored mind ; extremely fond of music and a very good musician, he was always a pleasant and interesting companion. Some idea of what was thought of him in Germany may be gathered from the following letter, received this week from Professor EHRLICH :—

[*Translation.*]

MY DEAR COLLEAGUE.—I have learnt with the greatest sorrow of the death of our common pupil and friend, MYERS. It is, indeed, a great loss—such an able, strenuous, trustworthy worker, and of so good a character. When you receive more detailed particulars from Dr. DURHAM, may I ask you to kindly communicate them to me ?

When the Liverpool School of Tropical Medicine was sending out the expedition, DURHAM and MYERS were approached to see whether they would take charge of it, and, though both of them fully appreciated the dangers to which they would be exposed and the risks they ran, they decided that they would take these risks and do the work that came to their hand. MYERS' death adds another name to the roll of martyrs to scientific investigation. His loss will be greatly felt by his many friends in Cambridge, and most by those who knew him best, for only those could appreciate to the full his fine and sterling qualities.

Immediately on receipt of the news the Committee of the Liverpool School of Tropical Medicine called a special meeting at which the following resolution was passed :—

Resolved—

'That the Liverpool School of Tropical Medicine desires to place on record its heartfelt sympathy with the family of the late Dr. WALTER MYERS in the very sad loss that they have sustained by the premature death in Para of that distinguished scientist in the discharge of a noble duty.

The School wishes to acknowledge in a public manner the high courage and the unselfish spirit that prompted the late Dr. MYERS unhesitatingly to accept the invitation of the School to take part in a most dangerous expedition, the principal object of which was the investigation of yellow fever, to which fatal disease Dr. MYERS fell a victim.

The School trusts that his family may derive some consolation from the fact that Dr. MYERS lost his life on behalf of humanity and science.'

The question of raising a suitable memorial to Dr. MYERS was at once taken in hand by the Committee, the result of which will be made public later. In the meantime the Committee have made offers, both of which have been accepted, to erect a tombstone over Dr. MYERS' grave in Para, and to present to the University of Birmingham, since he began his medical studies in Birmingham, a suitable brass memorial in his memory.

THE PREVENTION OF MALARIA IN TROPICAL AFRICA

By S. R. CHRISTOPHERS, M.B. Vict.

Since the discovery by Ross that malaria is a disease transmissible by the mosquito, many new facts bearing upon the prevention of malaria have come to light. Investigations carried on by Grassi, Celli, Bignami, and others in Italy, by Koch in New Guinea, by the Malaria Commission of the Royal Society and Colonial Office, by the expeditions of the Liverpool School of Tropical Medicine in Africa, have furnished us with a considerable amount of information concerning the life-history and distribution of mosquitoes, and on the nature of human malaria generally. As a result of these investigations many methods of combating malaria have suggested themselves, and have even been applied in several instances with success. The destruction of the larvae of anopheles, though a very obvious method of combating malaria, is by no means the only one. Other methods suggested by further researches are now acknowledged by many to be more easy of application and more effective. The conditions under which malaria occurs differ much, and under these different conditions widely different means of prevention may be found to be effective. Certainly many means, which in Europe are to be commended, must in Africa be very difficult to carry out, if not quite impossible.

The elucidation of a fact hitherto quite unexpected, namely, that the African native is not free from malaria, but is affected to an extraordinary degree, makes the prophylaxis of malaria in Africa a problem quite distinct from that of the prevention of malaria in Europe. A fundamental difference in the conditions in Africa and Europe at once presents itself. In Europe what is desired is to prevent malaria among the entire population. In Africa it is an evident impossibility to make any impression on malaria among a vast population, whose want of amenity to control can only be appreciated by those who have had actual experience of these people. In Africa what must be aimed at is the prevention of malaria among Europeans. This in itself will be a sufficiently great task to accomplish. The European is but one among many thousands of natives; it is in tiny settlements scattered over an enormous area that Europeans are for the most part to be found in Africa. Even in the few large towns the Europeans are but a few in comparison with the great bulk of natives. Thus a town of 30,000 or 40,000 inhabitants does not contain more than at most 150 Europeans.

Before discussing in detail the special methods of prevention which promise success in Africa, it will be well to briefly pass in review the main lines upon which malaria may be combated.

There are three main directions in which we may hope to influence malaria :

1 To attempt to destroy anopheles, either by doing away with the pools they breed in, or by destroying the larvae or adults.

2 To attempt to so protect men that anopheles cannot infect them, as by the use of mosquito nets or mosquito-proof houses.

3 To attempt to destroy the parasite in man himself, so that even though anopheles are present malaria is still absent.

The first and second methods appear at first most likely to lead to success. Recent researches, however, have shewn that the third method is a most effective way of dealing with the problem.

It may be pointed out that it appears to have been in this latter way that malaria has disappeared from the fen districts of England. Anopheles are still widely distributed, but there is no malaria. The probable explanation is that extensive use of quinine by the inhabitants of these districts has led to this result, and that the method Koch employed of combating malaria in New Guinea by quinine was unwittingly the cause of the disappearance of malaria in England.

I. THE DESTRUCTION OF ANOPHELES

The Superficial Drainage of an Area. Such a method can evidently be applied only to very limited areas. Even in Europe it would be difficult of application apart from towns, whilst in Africa there can be no question of its application except under certain special conditions. Even in most African towns drainage would be by no means a light task. In Lagos draining or filling up possible anopheles pools would be a gigantic undertaking. In Accra, situated on porous soil with an extremely scanty rainfall, it would not be so difficult. Even in Accra, however, it would probably only be possible after a water supply had been brought from the Aburi hills, for at the present time many of the inhabitants depend for water upon the very pits and wells which act as breeding-places. In Freetown, which is built on rock, the central parts of the town might be rendered free from all pools, yet the outskirts could not be so treated without great outlay.

Surface drainage in Africa must then be considered essentially of limited application. It is, indeed, only feasible when applied to a segregated community of Europeans. Such a special use of drainage is evidently by no means the enormous operation involved in draining a town of many thousands of inhabitants.

The Application of Culicicides. Great expectations were at one time held as to the efficacy of this method. In Africa the result of its application has not been

encouraging, and the destruction of anopheles by this means over an area of any size in Africa is by no means probable. It soon became evident that, in the rains and in swampy districts, anopheles breeding-places were so numerous, so widespread, and often so easily overlooked that little could be done under these circumstances. It still seemed possible that something might be done in the dry season, when the number of pools was often greatly reduced, to influence the number of anopheles in the ensuing rainy season. This might have been the case were it not that the life of anopheles is often a very lengthy one, and that anopheles often remain months without any possibility of their having recourse to water for breeding purposes. Late in the dry season prolonged search may be necessary before any breeding places are discovered in a district, and indeed large areas may not contain a single breeding-place. In spite of this adult anopheles are usually to be found in native huts throughout the district. The fact is that many thousands of anopheles, which have not originated in any pool existing at this time, have remained over (hibernating?) months after the pools they have bred in have disappeared. In spite, then, of the, at first sight, promising conditions during the dry season, it is doubtful whether at this time it is possible to influence the number of anopheles in the ensuing rains.

Were it possible generally to make quite sure that *all* pools in a district were regularly treated with culicicides, and were the process kept up for six months or a year, it is probable that the results would be most pronounced, and that malaria would be much diminished. Unfortunately, in practice such thoroughness is found to be very difficult and, out of Europe, almost impossible.

The Destruction of the Adult Insects. The adult insects can be attacked during the day by fumigation of houses. This process carried out systematically must be a great adjunct in any scheme of destruction of anopheles. It is a method, however, which is only adapted to Europe, and very little could be done in this way in Africa.

All the methods of combating malaria by destroying anopheles form a powerful means of prophylaxis where considerable labour can be commanded and where the area to be rendered free from anopheles is not large. They are quite useless in the prevention of malaria in the most deadly of conditions, namely, the up-country stations of Africa. As a whole, methods of this class are much more suited to Europe than to Africa.

II THE PROTECTION OF MAN FROM BITES OF ANOPHELES

As a means of prophylaxis this group is of the greatest importance.

The experiment carried out by Drs. SAMBON and LOWE has shewn that enthusiastic men may, in a mosquito-proof house, so effectually exclude anopheles as to live in a malarious district without contracting the disease.

Unfortunately, the effectiveness of these means depends almost entirely upon the person employing them. The majority of men in the tropics, either because

they do not appreciate the danger of being bitten, or from mere carelessness, are unwilling to take any trouble to avoid the bites of anopheles. It is, therefore, usual to see mosquito nets used in such a way that they afford but slight protection; whereas by the use, with scrupulous and unremitting attention, of a mosquito net a careful man may remain years in a malarial district without contracting malaria. It is also highly improbable that many men in Africa would so effectually exclude anopheles from a mosquito-proof house as the investigators on the Roman Campagna did. Indeed, it is highly probable that mosquito-proof houses would be as useless in the hands of most men in the tropics as mosquito nets are at present. Methods of protection from bites of anopheles are of great efficacy to those who are content to undergo some discomfort to avoid malaria, but of very little use to the average man who as yet protects himself at best only half-heartedly.

Mosquito Nets. As a means of protection the proper use of a mosquito net stands pre-eminently alone. Anopheles in most places do not bite by day, and as a rule are not very bold, so that they do not bite freely until the lights are out. By the careful use of a net the bites of anopheles may be almost done away with.

Clothing. A great deal of protection is afforded by the habitual use of certain precautions of dress. It is rare whilst a light is in the room for the face and hands to be bitten by anopheles. The parts usually bitten whilst one is still outside the net are the ankles, which are usually freely exposed, covered only by a single thickness of sock. By the use of double socks, mosquito boots, or puttees the ankles may be perfectly protected.

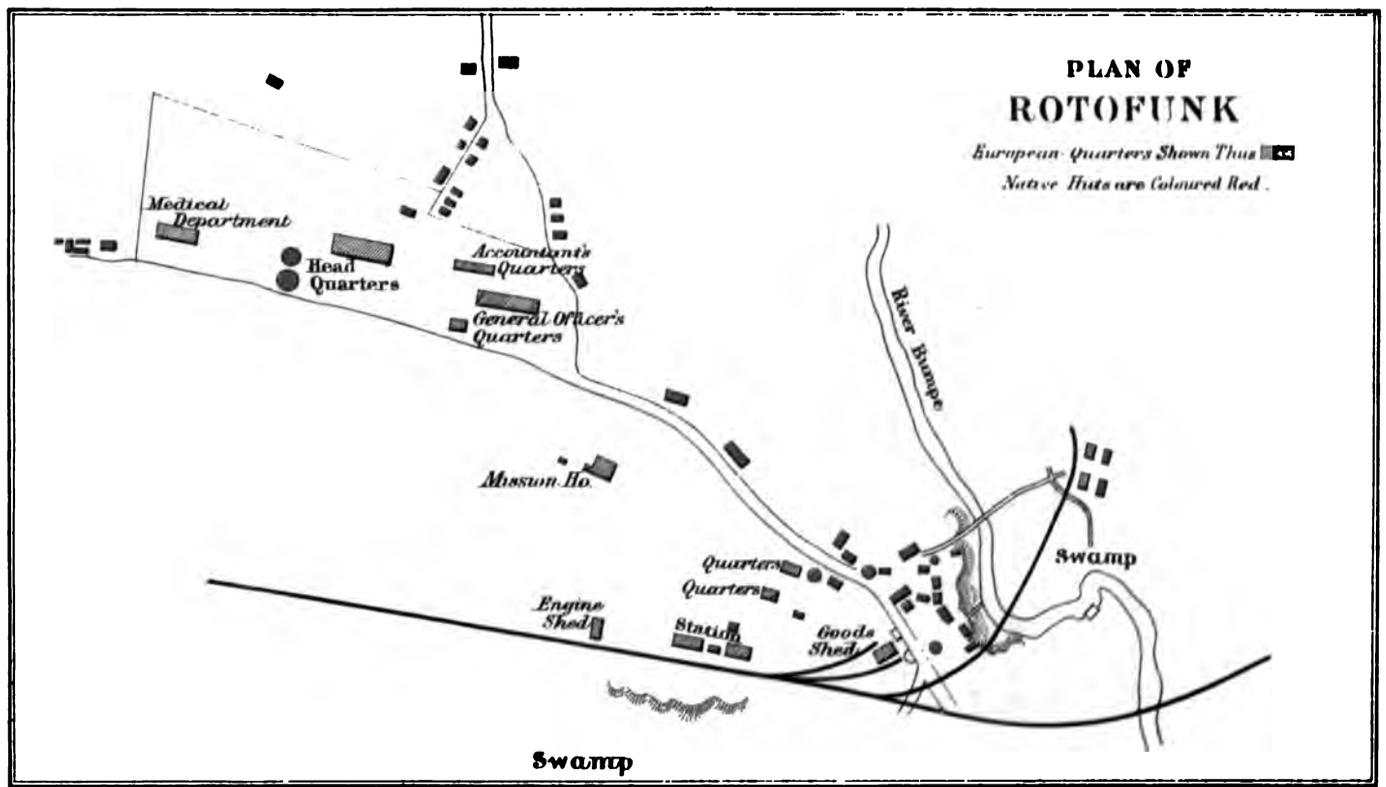
Mosquito-Proof Houses. As mentioned above mosquito-proof houses have been shewn to be feasible in Italy. It is, however, unlikely that such a means of protection will be largely used in the tropics. The climate in Italy and Africa are entirely different. In houses such as Drs. SAMBON and LOWE occupied, life in the tropics would be most unpleasant. With the most perfect ventilation, with doors and windows thrown open, it is often so excessively hot at night that sleep is difficult. Most Europeans occupy rather large and roomy houses and spend much of their spare time on the verandah. Protection of this kind we shall see also later is by no means the most promising method of preventing malaria among Europeans in Africa.

Repellant Bodies. So far no repellant body of any effectiveness has been discovered.

III. THE ELIMINATION FROM A COMMUNITY OF THE SOURCE OF INFECTION IN MAN

Two most important methods have the above result as their aim. They are:

1 The method employed with success by Professor KOCH in New Guinea, *viz.*, to search out all cases of malaria (the concealed ones in particular), and to render them harmless by curing them with quinine.


PLAN OF
ROTOFUNK
European Quarters Shown Thus
Native Huts are Coloured Red.
Medical Department
Head Quarters
Accountant's Quarters
General Officer's Quarters
Mission Ho.
River Bumpe
Swamp
Quarters
Quarters
Engine Shed
Station
Goods Shed
Swamp
G. Philip & Son.

In order to combat malaria he, at Stephansort, where malaria was rife, sought out all cases of malaria and persons with parasites in their blood, and treated them systematically with quinine.

By this means he was able to greatly reduce malaria in the on-coming season at Stephansort, although the climatic conditions were particularly favourable for the development of the disease. KOCH, in his fifth report to the German Imperial Health Bureau, sums up as follows: 'The results of our experiment, which has lasted nearly six months, have been so uniform and unequivocal that they cannot be regarded as accidental. We may assume that it is directly owing to the measures we have adopted that malaria here has in a comparatively short time almost disappeared.'

In a community then which can be kept under observation and systematically treated with quinine, malaria can be combated very effectively. In many parts of the world this method very probably will be the way in which the desired diminution of malaria will be brought about. In Africa, however, such a method is quite inapplicable, and it would be quite impossible to carry it out under existing conditions, even in the most limited way.

A second method of getting rid of the source of infection is, however, peculiarly adapted to Africa.

2 The method first recommended by the Malaria Commission, and later strongly advocated by the expedition of the Liverpool School to Nigeria, *viz.*, segregation of Europeans from Natives.

This method is a corollary of the discovery that native children in Africa practically all contain the malaria parasite and are the source from which Europeans derive malaria.

KOCH shewed in New Guinea that in most places infection was very prevalent in native children, so much so that in some villages 100 per cent. of those examined contained parasites. He also shewed that as the children increased in age immunity was produced, so that in the case of adults a marked immunity was present and malarial infection was absent.

The Malaria Commission shewed independently that a condition of universal infection existed among the children of tropical Africa, associated with an immunity of the adults. This infection in children had many remarkable characteristics. The children were in apparent health, but often contained large numbers of parasites, and a small proportion only of the children failed to shew some degree of infection. Not only were the children in every village and every hut infected, but anopheles were found which, from feeding on the children, had become infected.

It became evident that European malaria in Africa was but a mere indication of an enormous degree of infection throughout the whole of the malarious regions of Africa; and that the clue to the prevention of malaria among Europeans in Africa

lay in the appreciation of this previously unsuspected and enormous source of infection.

The Liverpool School Expedition found a similar condition of affairs in all parts of Nigeria visited by them.

With a knowledge of the ubiquity of native malaria, the method of infection of Europeans becomes abundantly clear. The reputed unhealthiness or healthiness of stations is seen at once to be dependent on the proximity or non-proximity of native huts. The attack of malaria after a tour up country, the malaria at military stations like Prah-su, the abundance of malaria on railways, are all explicable when the extraordinary condition of universal native infection is appreciated.

It is evident that could Europeans avoid the close proximity of native huts they would do away with a very obvious and great source of infection. That they could avoid the neighbourhood of huts no one who has studied the conditions of life in Africa can doubt. On the other hand European houses in tropical Africa are almost always to be found with several native huts close at hand. When it is understood that each of these huts certainly contains many children with parasites in their blood, and also scores or hundreds of anopheles to carry the infection, then the frequency with which Europeans suffer from malaria is scarcely to be wondered at.

Sometimes the huts are those of the Europeans' servants, and here a mere command would be sufficient to ensure, in a day or two, freedom from such sources of infection. In other cases the huts are beyond the control of the European : but here again had the site of his house been chosen with a view to health, and situated away from huts originally, malaria would have been avoided. Throughout Africa Europeans are suffering from constant and repeated attacks of malaria, which owe their origin entirely to the presence of these huts, and which might with infinitesimal trouble be avoided.

SUMMARY

To sum up then the application of these methods to conditions in Africa.

1 The destruction of larvae is from the magnitude of the task only applicable in special conditions.

2 The methods of protection from mosquito bites are efficient only when great personal care is used. These means necessitate the most vigilant and unremitting attention, and are therefore more a matter for the individual than a community.

3 (*a*) The seeking out of cases of malaria, or any drug treatment of the natives, is quite impossible in Africa.

(*b*) The separation of Europeans from the close neighbourhood of any native hut is a way in which Europeans may, without constant worry and trouble, protect themselves from malaria.

It is a method, moreover, which is peculiarly adaptable to the very conditions under which other methods are most futile and malaria most deadly, namely, the small settlements scattered about the African colonies.

Up to the present, segregation has not received the attention which its simplicity and practicability deserve. It is significant, however, that the two latest expeditions to Africa, namely, the Malaria Commission and the Liverpool School Expedition, are unanimous in upholding it as pre-eminently above all other schemes of prophylaxis as applied to Africa. No doubt the absence of attention is partly due to an insufficient appreciation of the condition of universal native infection, and partly to a want of knowledge of the conditions under which Europeans live in Africa.

It has been objected that segregation is impossible to the trader and commercial section of the community, and that the natives would by its application be apt to take offence. Both these objections are based upon a mistaken idea as to what it is proposed should be done to diminish malaria in Europeans by segregation.

As it is essential that the conditions of life of Europeans in Africa should be first thoroughly understood, a brief description of these conditions as most commonly seen is desirable.

1 *Up-country Stations.* Whether Government official quarters, missionary or traders' quarters: the Europeans' house may or may not be a well-built dwelling adapted to the climatic conditions. In either case there is always:

(*a*) A palm or grass hut or more pretentious building which is nominally the kitchen, but which is also used by a variable number of native servants and often by their families as a sleeping place. This structure is always within a few yards of the house.

(*b*) On some pretext, or for no assignable reason, there are always a few huts or a small village close at hand. This condition is universal, and the practice is responsible for the malaria from which Europeans in Africa suffer.

(*c*) Unlimited ground quite free from huts and easily attainable.

Here it is evident that there is nothing to prevent the European living in comfort and health.

The kitchen is essential; not so its use by all the native servants as a sleeping place. It is doubtful, indeed, whether any servant is necessary at night in most instances. Certainly many servants in the towns go to their homes and return again in the early morning. Were even one servant considered a necessity at night there need be no more, and there certainly need not be any children allowed. In a day or two the servants would build new quarters at any distance if so ordered.

The native huts of the village should have been avoided in the first instance, and, indeed, for a house to be placed close to a village can be looked upon now only as a folly. In many cases the European has it in his hands to move to a less dangerous neighbourhood, or even to get the huts themselves done away with.

By such simple means would malaria be successfully combated. By such means stations now deadly with malaria might be rendered healthy. Not only is it the case that quarters already erected may be improved, but quarters not yet erected may be made healthy or unhealthy as they are erected with the principle of segregation in view or with the principles which have hitherto prevailed.

The accompanying plan is that of a new railway settlement on the Sierra Leone railway. Miles of land free from huts exist along the line, but the close neighbourhood of native huts has been selected. At the time of building of these quarters it lay in the power of the engineers to have a malaria-free settlement. Instead of which, by the non-observance of a simple fact, the station is most malarious. In this particular instance much ingenuity has been shewn in providing each set of European quarters with plenty of malarial infection.

Before malaria is lessened among Europeans in Africa it must be generally recognised that malaria is practically an infectious disease, and that it is present in every native hut.

2 *A Town Residence.* In towns only is there any difficulty in carrying out the principle of segregation. In two instances, however, this has been carried out in towns with the result that the segregated communities of Europeans are notoriously the most healthy on the West Coast.

Even when no scheme of complete segregation can be carried out, the principle should always be borne in mind, and, whenever opportunity offers, huts should be removed and European houses built in the open. Possibly segregation will be even more beneficial in dealing with new settlements and new towns than with those already built among bad surroundings.

One thing is certain, that no scheme but segregation offers the least promise of a wide success in Africa.

ENLARGED SPLEENS AND MALARIA

By C. W. DANIELS, M.B. Cantab., British Guiana Medical Service

Enlargement of the spleen is so much more common in malarial countries than elsewhere that the dependence of this condition on malaria can hardly be doubted. The exact relation of the two conditions is, however, obscure.

In India the 'Spleen Test,' *i.e.* the proportion of unselected persons who are found to have obvious enlargement of the spleen, has by many been relied on as *the* test of the prevalence of malaria in a district.

Elsewhere, probably, as will be shown, owing to working with a greater variety of races, difficulties have been met with in placing reliance on this view. British Guiana is certainly a more malarious country than most parts of India, yet in a series of two hundred and twelve post-mortem examinations made in the Berbice Lunatic Asylum, 1881-1883 (*Asylum Journal*, Feb., 1883), recorded by Dr. Grieve, it was shown that a larger proportion of spleens were below than above the normal range of weight. Of these two hundred and twelve, one hundred and twenty-three or 58 per cent. were between four and ten ounces, *i.e.* were about normal; fifty-five or 26 per cent. were under four ounces, or were subnormal; and only thirty-four or 16 per cent. were over ten ounces. Even of these thirty-four, ten were from ten to twelve ounces, and only five or 2·3 per cent. over twenty ounces, markedly enlarged. These patients were adults, and the majority were probably negroes, but the returns are not sub-divided according to race or age.

From 1893-96, some eighteen hundred post-mortem examinations were made, and the presence or absence of malarial pigment was determined in each case by microscopical examination.

The result, as shown in the subjoined table, up to 1895, showed that there was no fixed relation, except in cases of acute malaria, between the weight of the spleen and the presence of malarial pigment in that organ; and further, showed that great differences existed in the size of the spleen in different races, whilst there was comparatively little difference in the prevalence of pigmentation.

The results were similar in males and females; but, to avoid repetition, the tables for adult males only are given.*

*Fuller particulars of this portion of this series are given in the *British Guiana Medical Annual for 1895*, Appendix to 'Notes on a series of Post-mortem Examinations.'

TABLE I

Race	East Indians		Negroes Natives B.G.		Aboriginals		Chinese		Portuguese		Immigrant Negroes		Europeans	
	Not Pigmented	Pigmented	Not Pigmented	Pigmented	Not Pigmented	Pigmented	Not Pigmented	Pigmented	Not Pigmented	Pigmented	Not Pigmented	Pigmented	Not Pigmented	Pigmented
Number of Cases . . .	345	80	171	57	9	4	19	6	37	3	65	63	12	7
Percentages, 5 ozs. & under	4·9	11·2	33·9	35·2	...	...	5·2	16·6	5·4	...	46·1	31·9	25·5	...
„ 10 ozs. „	18·5	32·5	47·3	42·6	22·2	25	36·8	33·3	30	33·3	32·3	41·2	41·6	...
„ 15 ozs. „	24	23·7	9·9	14·8	11·1	...	15·7	33·3	16·2	...	6·1	14·2	8·3	57·1
„ 20 ozs. „	15·6	20	6·4	5·5	22·2	50	21	16·6	24·3	...	12·2	6·3	16·6	14·2
„ 25 ozs. „	12·7	2·5	1·2	...	11·1	...	...	...	8·1	...	...	4·7	...	14·2
„ 30 ozs. „	9·8	2·5	·58	1·9	22·2	...	5·2	...	8·1	...	...	...	...	14·2
„ 40 ozs. „	5·2	5	·58	...	...	...	15·7	...	2·7	66·6	...	1·6	...	...
„ 50 ozs. „	5·5	...	...	...	...	25	...	...	2·7	...	1·5	...	...	...
„ 60 ozs. „	2	...	...	...	...	...	...	...	...	...	...	...	...	...
„ over 60 ozs.	2	2·5	...	...	11·1	...	...	...	2·7	...	...	...	...	..

Of the races represented, the Aboriginal American Indians, the Chinese, and Portuguese are represented in too small numbers to be of much value in themselves, but it is seen that they correspond to the Indian type and not to the Negro.

The two last columns differ from the others in that they represent races exposed to malarial infection in adult life only, such as the Immigrant Negroes from Barbados and other non-malarial West Indian Islands.

The age incidence amongst adults is represented in the following table; but at these ages, twenty and over, recent malarial attacks are rare as shown by the rarity of recent malarial pigmentation, and therefore this table does not show the effects of recent attacks.

TABLE II

Race		E. Indian		Native Negroes		Immigrant Negroes	
Ages		Not Pigmented	Pigmented	Not Pigmented	Pigmented	Not Pigmented	Pigmented
20-25	10 oz. and under	33·3	49·9	75	71	66·6	47·5
	Over 10 oz.	66·6	50·1	25	28·9	33·3	52·5
26-45	10 oz. and under	17·9	39·6	86·4	80	85·4	79·9
	Over 10 oz.	82·1	60·4	13·6	20	14.6	19·9
Over 45	10 oz. and under	37·4	60	81·9	92·2	74·5	100
	Over 10 oz.	62·6	40	18	7·6	25·5	—

In younger persons the pigmented organs are much heavier than the unpigmented organs; so that at these ages the spleens which show pigmentary evidence of malaria are also enlarged.

In British Central Africa post-mortem examinations were not obtainable, but clinical observations were made. Amongst the native adults I did not see a single case of enlarged spleen, though several hundred examinations were made; but I was informed by others that the condition, though rare, did occur.

In native children, however, enlarged spleen was common. The variation in the age incidence was marked, and differed also in different districts according to the liability to malarial infection as determined by the length of residence requisite for infection of susceptible newcomers. In districts represented in the first vertical column of Table III, a few of the newly-arrived Europeans escape 'fever' for a year or more; in those represented in the second column infection is usual in a month, and few persons escape much longer; whilst in those represented in the third column an exposure for a fortnight or so results in the infection of nearly 25 per cent. of the Europeans exposed.

TABLE III

ENLARGED SPLEENS IN PERCENTAGES

	Highlands 3,000 feet or more	Lake Level 1,500 feet odd	Lower Shiré 300 feet or less
Aged two years and under -	18·4	18·8	48·1
Two to four years - - - -	31·5	45·1	57·1
Fifteen years and under - -	26·2	11·5	13·7

The total number of these children was eight hundred and fifty. Of these five hundred and thirty-five were fifteen and under; one hundred and twenty-four were two to four years old; and one hundred and ninety-one were under two years. The ages in only a portion of the cases could be determined with certainty, in the others it had to be estimated from the development.

Attacks of 'fever' are common in cachectic or persons with enlarged spleens. In these attacks it is exceptional to find malaria parasites; pigment is usually absent from the organs in fatal cases, and the action of quinine is uncertain.

Persons with chronic enlarged spleens, with or without cachectic, are peculiarly liable to certain diseases, particularly lobar pneumonia.

As regards Europeans, the fact that few are in continuous residence in badly malarial places for long periods renders such questions very difficult to consider. Even with negroes there is some reason to suppose that their acquired immunity is diminished by residence in a non-malarial country. If in an ordinary attack of untreated malaria the parasites be counted in successive cycles, the number is not as a rule increased in the second or third observed cycles, and is often diminished. When working with MAJOR Ross on *Proteosoma*, I was able to confirm his observation on birds infected by mosquitoes, to the effect that the number of parasites found at first rapidly increased, then remained stationary for two or three days, and finally rapidly diminished to such an extent that, whilst at the height of the invasion eight or nine parasites might be found in a field, later a single parasite only would be found in a slide on one day and none on another.

These observations show that there is a check on the multiplication of the parasites, as otherwise they would increase in geometrical progression. Natural cure is common, and the parasites disappear or occur in such small numbers that they are not found in blood examinations, and leave no deposit of pigment in the organs.

In many of these cases it is probable that a small residue of parasites is left, as in such cases recurrence of the parasites takes place without fresh infection. This interval between attacks is one of temporary immunity. It may be long or short, but in newcomers is usually under a month. It varies in different individuals, and in the same individual at different times.

In a place, or even in a house, where frequent fresh infections are occurring, the period of immunity may be of several months after a single attack of fever. In one station, few newcomers escaped fever for more than a month after arrival ; but the older residents did not have attacks more frequently than every three, four, or six months or more. In this place, with the older residents, infections must have been more frequent than actual attacks of fever ; so that several of the infections must have been abortive, indicating a degree of immunity in these persons.

A common history amongst the older European residents was repeated attacks of fever in the first two, three, or four years, and very little or only slight attacks subsequently. Many of these persons were not cachetic, and had no splenic enlargement. In the temporary periods of immunity the person often appears to be in robust health.

The loss of acquired immunity by change of residence to a non-malarial country would, if fully established, indicate that, though immunity to malaria is acquired by repeated invasions of the parasite (*i.e.* relapses), repeated fresh infections, however abortive, are required for its maintenance.

Under these circumstances a high degree of immunity is not likely to be attained in countries or districts where for a lengthy portion of each year fresh infections do not occur.

In the malarial countries in which I have worked, though there has been a seasonal variation, there has been no period during which fresh infections have not occurred.

As a test for the prevalence of malaria, the 'spleen test' may be worse than useless unless race and age are taken into account. In Africa, if examination be made of persons five to fifteen years of age, the least healthy districts would appear to be the least malarial, judged by the proportion of enlarged spleens. If adult natives alone were examined all districts would appear to be free from malaria.

In South America malaria would appear to be rare or common in a district according to the race, Negro or Indian, examined.

Even in a country with a population of uniform race, examined at an age at which splenic enlargement is most common, we known too little of the other factors causing enlargement of the spleen for the test to be at all an accurate one.

It must always be remembered that even such characteristic sequelae of diseases as neuritis after diphtheria, nephritis after scarlatina, and organic cardiac valvular disease after rheumatism, and even stricture after gonorrhoea are dependent on other factors as well as on the primary disease; and the frequency of the sequela would afford no real measure of the prevalence of the primary disease. Excluding the factors, race and age, splenic enlargement can be considered as a rough inaccurate measure of the prevalence of malaria, but still a useful one.

DIAGRAMS ILLUSTRATING THE LIFE-HISTORY OF THE PARASITES OF MALARIA*

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With Plates

The parasitology of the red blood-corpuscle of vertebrates was opened in 1870 by Ray Lankester's discovery of the Drepanidium ranarum. In 1880 Laveran made the important observation that somewhat similar intra-corpuscular organisms exist in the blood of human beings suffering from malarial fever. Since then Danielewsky, Kruse, Koch, Dionisi, and others have demonstrated allied parasites in the blood of reptiles, birds, bats, and monkeys, and Smith and Kilborne have shown that the disease of oxen called Texas cattle fever is due to an intra-corpuscular parasite of another kind. As the result of these observations we are now familiar with a considerable number of such organisms. All of them are usually classed among the protozoa, and in the somewhat artificial order of the sporozoa. They are generally divided into three groups, which are as follows :—

Group I The parasite of Texas cattle fever, Pyrosoma (or Apiosoma) bigeminum, Smith and Kilborne, and similar organisms found in dogs and some other mammalia (?) ; minute pear-shaped intra-corpuscular bodies, which are known to be communicated among oxen by the cattle tick, Boöphilus bovis.

Group II Organisms apparently allied to the Gregarinidae; found in reptiles; numerous species.

Group III Intra-corpuscular amoebae or myxopods found in man, monkeys, bats, birds, and possibly frogs. Four species are known to undergo further development in gnats.

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It is this last group which claims our attention at present. The diagrams are meant to illustrate a discourse delivered by one of us at the Royal Institution (*Proceedings of the Roy. Inst.*, 1900, and also *Nature*, March 29, 1900); but we shall now give a description sufficient to enable the reader to follow the life-history from this paper alone. The figures show the appearance of the parasites as seen in unstained preparations—the cytology of some of the stages (Figs. 53-60) in the gnat not yet being sufficiently established to warrant illustration in a scheme of this kind.

We adopt the name Haemamoebidae, WASIELEWSKY, for the whole group. At least three species occur in human beings (producing the different varieties of malarial fever); one species in monkeys, three in bats, and two in birds. We illustrate only the species found in man and birds, those of monkeys and bats being closely similar to the human species, but, so far as we know at present, not identical. The development of four of the species has been followed in gnats. The three human species develop in gnats of the genus Anopheles, while one of the avian species (Haemamoeba relicta) lives in gnats of the Culex pipiens type. The insect hosts of the remaining species have not as yet been found.

So far as we know, the life-history of all the species is practically identical, and is as follows :—The youngest parasites are found as minute amoebulae living within or upon the red corpuscles of the vertebrate hosts. Each contains a nucleus, which stains by the ROMANOWSKY method. Growing rapidly in size, the amoebulae convert the haemoglobin of the containing corpuscles into a varying number of brown or black granules, which are called the melanin or malarial pigment. These granules lie in the bioplasm of the parasite surrounding the nucleus. After an interval of from one to several days (according to the species concerned) the amoebulae, still contained within the corpuscle, reach maturity, and become either (*a*) sporocytes or (*b*) gametocytes. In the case of the amoebulae which become sporocytes the nucleus divides into a number of segments (varying according to the species). Each segment of the nucleus surrounds itself with a portion of the bioplasm, and becomes a spore—the process being obviously one of simple asexual propagation. Finally the corpuscle which contains the parasite, and which has now been almost entirely destroyed by it, bursts and liberates the spores, allowing them and a small nucleus de reliquat, consisting chiefly of the melanin, to fall into the liquor sanguinis. The melanin is taken up by the phagocytes of the host, while the spores attach themselves to fresh red corpuscles, become amoebulae in their turn, and thus continue the life of the organisms indefinitely within the vertebrate hosts.

In the case of the amoebulae which become gametocytes the history is quite different. It is not yet definitely known what determines a given amoebula to become either a sporocyte or a gametocyte, but the fact must be accepted. In the gamoetocytes the nucleus does not divide as in the case of the sporocytes—the parasite reaches maturity without showing any sign of spore-formation. In the majority of

species (genus Haemamoeba) the gametocyte has a general form similar to that of the sporocyte before the spores are produced; but in one species (genus Haemomenas) the gametocyte has a special (crescentic) shape, which is recognisable at an early stage in its career. As their name indicates, the gametocytes are sexual forms, male and female. They possess no function within the vertebrate host, but are meant to continue the life of the organisms within a second host—a suctorial insect.

When the gametoyctes are drawn into the stomach cavity of gnats (middle intestine) they immediately undertake their sexual functions. The male gametocyte (the nucleus of which is larger than that of the female) is destined to give origin to a number of microgametes, or spermatoza; the female gametocyte develops into one macrogamete, or ovum, together with a residuum consisting chiefly of melanin. A few minutes after ingestion by the gnat both male and female gametocytes break from the enclosing corpuscle, and swell slightly. Attached to the naked parasite one can now often perceive one or two small spherical objects, which may possibly be the homologues of polar bodies. A few minutes later a quivering movement is observed in the male gametocytes, due to the emission of the microgametes. These bodies are long filaments endowed with very active powers of locomotion, and consisting of a thread of chromatin surrounded by a thin scroll of bioplasm. Breaking away from the parent cell, and leaving behind the melanin of that cell as a residuum, the microgametes travel through the liquor sanguinis contained in the stomach of the gnat in search of a macrogamete. This being found, one microgamete enters the macrogamete and unites with its nucleus, producing a zygote.

Shortly after the act of fertilisation the zygote may in some species become motile (when it is technically called a vermicule), and generally changes its shape. At all events, it travels towards the parietes of the stomach. If the insect be of an inhospitable species the zygote perishes; but if the insect be hospitable the zygote passes through the parietes and affixes itself on or just under the outer muscular coat of the stomach. Here it becomes motionless and commences to grow rapidly in size.

At first of about the size of a red corpuscle, and still containing the characteristic black granules of melanin, the zygote, after a week or so, reaches a very large size; that is, it becomes about 60 μ in diameter, or about eight times its original diameter, and about five hundred times its original bulk. As we have said, we are not satisfied regarding the nature of the nuclear changes during the growth of the zygotes, but it is clear that the parasite acquires a very distinct capsule, and that its substance divides into from eight to twelve meres, which can easily be distinguished without staining. Each mere seems finally to become a spherical blastophore, bearing on its surface a number of filamentous, or rather spindle-shaped blasts, in the manner depicted in Fig. 60; at least, it is easy, by rupturing a nearly mature zygote, to expel a number of such bodies. When the zygote reaches maturity the blastophores disappear, leaving the capsule packed with thousands of the blasts (and containing also some residual fatty globules).

The capsule now bursts spontaneously, and pours the blasts into the body-cavity of the gnat. On drying and staining the blasts are easily seen to be of about 12 to 16 μ in length, with a central nucleus, one or two clear oval areas, and tapering extremities. No definite movements have been observed in these bodies—possibly on account of the reagent (salt solution) which must be used to make them visible in fresh preparations. By some means or other, however, they find their way into remote parts of the host, and finally pierce the capsule of its salivary gland, enter the salivary cells, and lastly the salivary ducts, in all of which situations they can easily be seen with the aid of a strong salt solution. From the salivary ducts they evidently pass through the insect's middle stylet or tongue into the circulation of a fresh vertebrate host, in which it is to be presumed they at once become the amoebulae with which the life-history of the parasites commenced. At all events, numerous experiments, both on birds and on man, have demonstrated the fact that gnats whose salivary glands contain the blasts are capable of establishing infection by their bites in the appropriate vertebrate hosts.

It should be noted that this life-history is in no way a hypothetical one. Every fact has been confirmed over and over again by many capable observers. The stages in the vertebrate hosts, first established by LAVERAN and GOLGI, have been scrupulously studied, and have given rise to a mass of literature already very large. The sexual functions of the gametocytes—which can be witnessed *in vitro*,—originally observed by MACCALLUM, have been seen also by KOCH and MARCHOUX ; while the facts are confirmed by the cytological studies of BIGNAMI and others. The development in the gnat and the infection of the healthy vertebrate host by the bite of the gnat, originally established by one of us, have been confirmed by DANIELS, KOCH, GRASSI, BIGNAMI, BASTIANELLI, and many others, and are accepted by LAVERAN, RAY LANKESTER, METSCHNIKOFF, MANSON, CELLI, and other distinguished men of science. A full history of the subject of the relation between malaria and gnats, together with a complete bibliography, has been written by NUTTALL.*

The terminology used above was arranged in consultation with Professor HERDMAN, F.R.S. It has been adopted by LAVERAN and MANSON. GRASSI and others had previously employed the terms gamete and zygote, but used sporozooid for the bodies we call blasts. We use the word blast somewhat in the sense employed in embryology, and as synonymous with sexually-produced spores. RAY LANKESTER suggests for them the name filiform young.

It should be added that, within the capsules of mature zygotes large black bodies (Fig. 67) are often to be found. It is doubtful whether these have any real connection with the parasites.

* Nuttall, *Hygienische Rundschau*, numerous papers, 1898, 1899, 1900. Also, *On the Role of Insects . . in the Spread of Diseases, Johns Hopkins Hospital Reports*, Baltimore, vol. viii.

The most recent figures of stages of the malaria parasites are those published by KOCH, *Zeitschrift fur Hyg. und Infect.*, 1899 (photographs) ; Grassi, Bignami, and Bastianelli, *Annali d'Igiene sperimentali*, 1899 (plates) ; *Sierra Leone Expedition, Thompson Yates Laboratories Report*, vol. ii. (photographs and drawings).

We divide the haemamoeba into two genera (*Nature*, August 3, 1899), namely :—

Genus I—Haemamoeba, GRASSI and FELETTI. The gametocytes have a shape like that of the sporocytes before the spores have been produced.

Genus II—Haemomenas, gen. nov. (syn. *Laverania*, GRASSI and FELETTI, in part). The gametocytes have a special (crescentic) shape.

DESCRIPTION OF PLATES

Illustrating Major RONALD ROSS's and Dr. R. FIELDING-OULD's paper on *The Life-History of the Parasites of Malaria.*

FIGS. 1-41 Illustrate the two avian species and the three human species in the blood-vessels of the vertebrate hosts.

FIGS. 42-52 Illustrate the development of the gametocytes of one of the human species, selected for the purpose, in the stomach cavity of anopheles, or in a drop of blood freshly extracted from the finger (in which the parasites develop much as they do in the stomach cavity of the insects).

FIGS. 53-67 Illustrate the further development of the parasites in the tissues of the gnat.

FIGS. 1-7 Development of Haemamoeba Danilewskii (syn. *Halteridium Danilewskii*, Labbé) in the blood of pigeons, jays, crows, etc. The amoebula is elongated, and lies by the side of the nucleus without disturbing its position. Several varieties exist.

Fig. 1 Spore.

Figs. 2-4 Growth of amoebula.

Fig. 5 Sporocyte.

Fig. 6 The containing corpuscle has burst, leaving nucleus, melanin, and free spores.

Fig. 7 Gametocyte.

FIGS. 8-14 Development of haemamoeba relicta (syn. *Proteosoma Grassii*, Labbé) in the blood of sparrows, larks, crows, etc. The amoebula lies at one end of the corpuscle, and pushes the nucleus towards the other end.

Fig. 8 Spore.

Figs. 9-11 Growth of amoebula.

Fig. 12 Sporocyte.

Fig. 13 Freed spores.

Fig. 14 Gametocyte.

FIGS. 15-22 Development of haemamoeba malariae, the parasite of quartan fever of man. The containing corpuscle is a small or medium-sized one, the melanin consists of coarse dark-brown granules, the amoeboid movements are slow, the spores are about eight to twelve in number, development takes seventy-two hours.

Fig. 15 Spore.

Figs. 16-18 Growth of amoebula.

Fig. 19 First appearance of spore formation.

Fig. 20 Sporocyte.

Fig. 21 Freed spores.

Fig. 22 Gametocyte

FIGS. 23-29 Development of haemamoeba vivax, the parasite of tertian fever of man. The containing corpuscle is nearly always an exceptionally large one, the melanin consists of very fine light-brown granules, amoeboid movements are rapid, the spores are about twelve to twenty in number, development takes thirty-six hours.

Fig. 23 Spore.

Figs. 24-26 Growth of amoebula.

Fig. 27 Sporocyte.

Fig. 28 Freed spores.

Fig. 29 Gametocyte.

Figs. 30-41 Development of haemomenas praecox, the parasite of remittent, pernicious, or aestivo-autumnal fever of man. Several varieties, which are possibly distinct species. The amoebulae are small, contain only a few clumps of black melanin, and retire to the spleen, bone-marrow, etc., on approaching maturity ; the gametocytes have a special crescentic shape.

Figs. 30-34 Growth of amoebula becoming a sporocyte.

Fig. 35 Freed spores.

Figs. 36-39 Growth of amoebula becoming a gametocyte.

Fig. 40 Male gametocyte (portion of red corpuscle still present).

Fig. 41 Female gametocyte (remains of corpuscle entirely disappeared).

Figs. 42-52 Development of gametocytes of haemomenas praecox in stomach cavity of Anopheles.

Fig. 42 Male gametocyte.

Fig. 43 Female gametocyte

Figs. 44-45 Gametocytes become oval five minutes after ingestion by the gnat.

Figs. 46-47 Gametocytes become spherical a few minutes later. Polar bodies appear at the margin.

Fig. 48 Male gametocyte emitting microgametes (the so-called 'flagellate body').

Fig. 49 Female gametocyte or macrogamete awaiting fertilisation.

Fig. 50 Free microgametes.

Fig. 51 Microgamete entering macrogamete.

Fig. 52 Fertilised macrogamete, or zygote.

Figs. 53-67 Development of Haemomenas praecox in tissues of anopheles.

Fig. 53 Zygote approaching inner surface of stomach wall.

Fig. 54 Zygote piercing stomach wall.

Figs. 55, 56 Zygotes affixed to outer coat of stomach. They possess an apparently alveolar structure, and still contain granules of melanin.

Fig. 57 Zygote increases in size.

Figs. 58, 59 Zygotes increasing in size and dividing into meres, which become blastophores bearing blasts.

Fig. 60 A single blastophore bearing a number of blasts affixed to it, each by one extremity.

Fig. 61 Stomach seen by a low power, and dotted with a number of mature zygotes.

Fig. 62 A fully mature zygote packed with blasts, some of which are escaping from a rupture in the capsule.

Fig. 63 Free blasts in body cavity of anopheles.

Fig. 64 Blasts entering capsule of salivary gland ; also lying within salivary cells and duct.

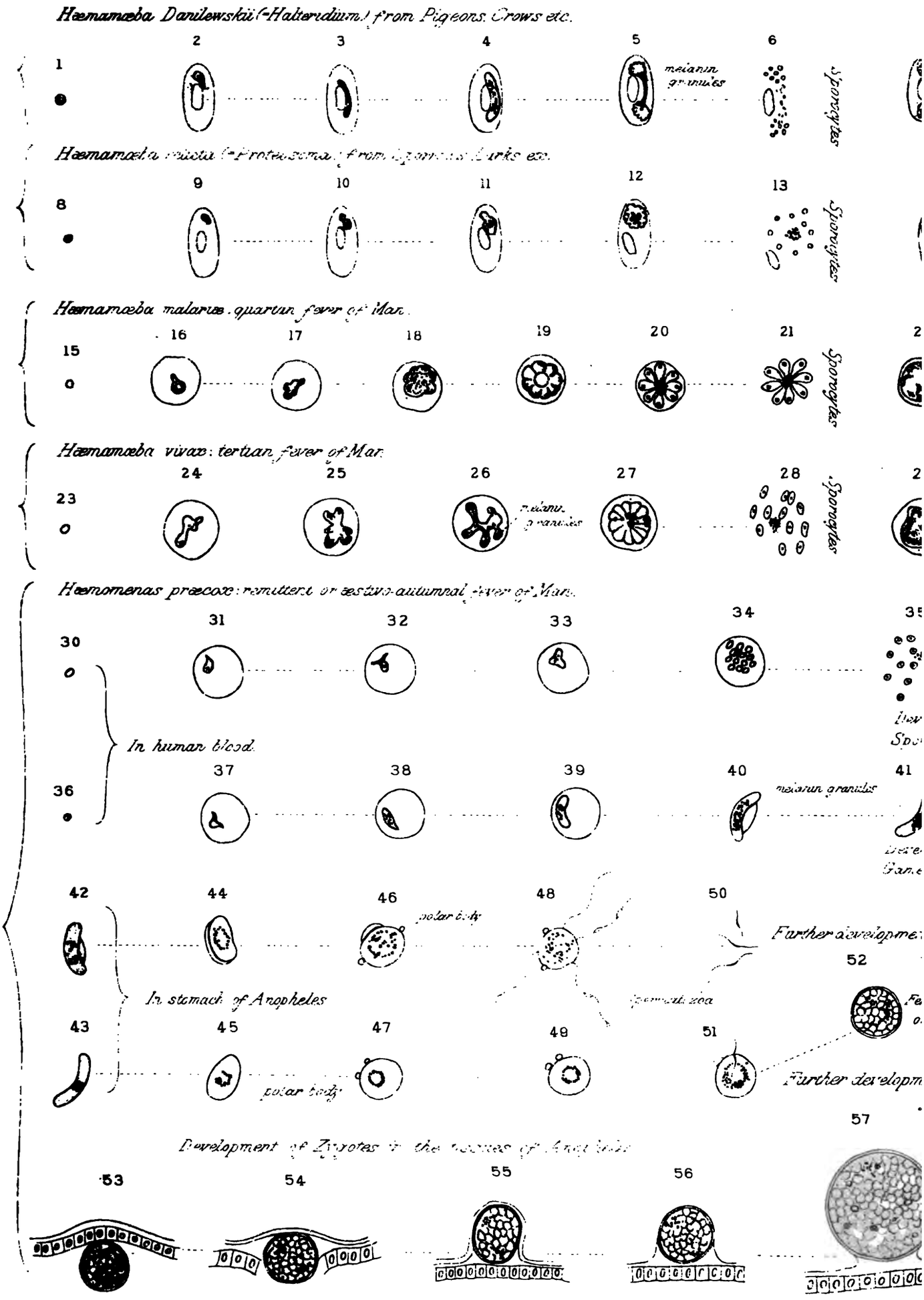
Fig. 65 Junction of ducts of three lobes of salivary gland of one side, with blasts.

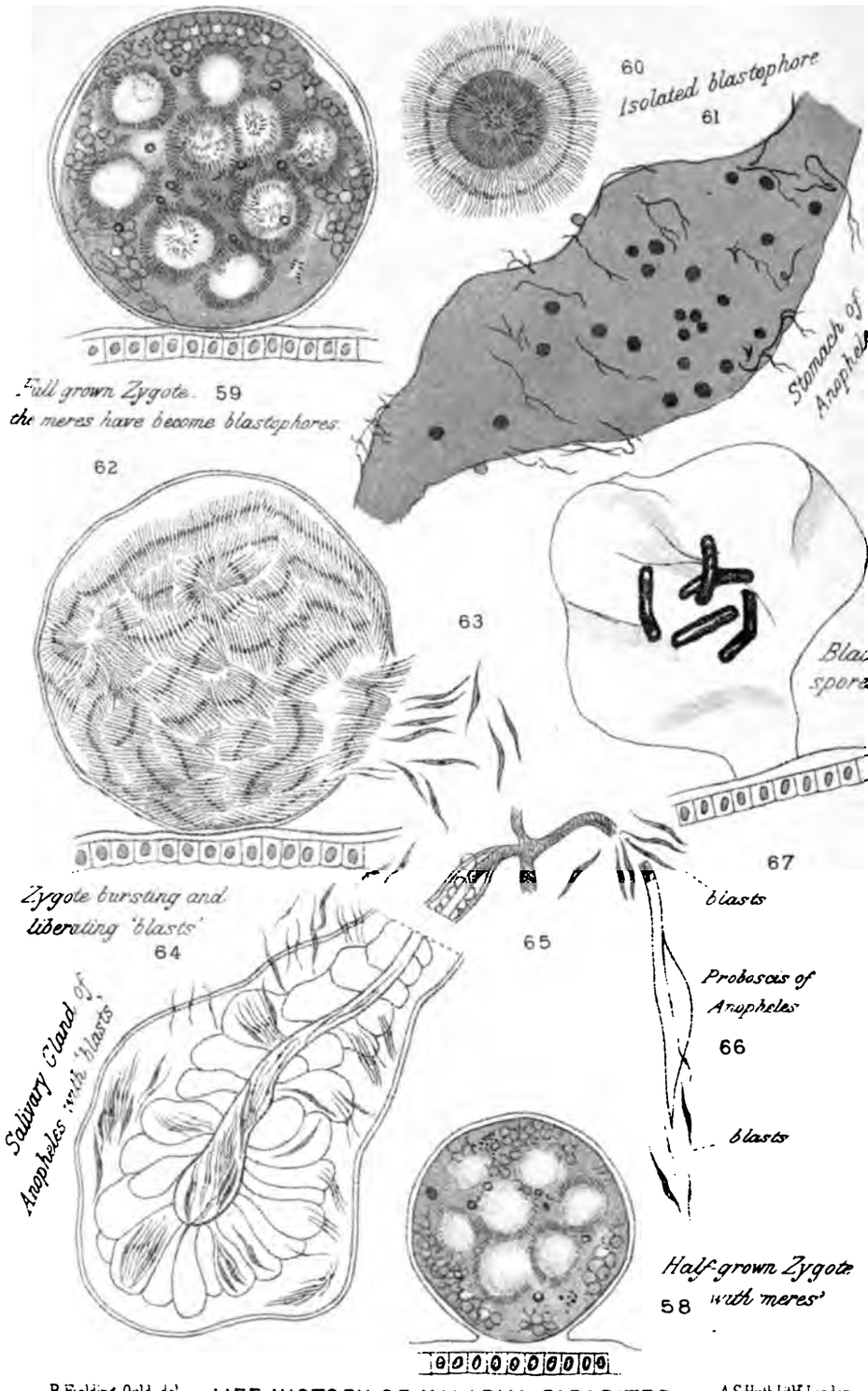
Fig. 66 Blasts escaping from extremity of middle stylet or tongue of anopheles.

Fig. 67 Capsule of a mature zygote containing five large black bodies, provisionally known as 'black spores.'

NOTE—We may point out that the development of the remaining human species in anopheles and of H. relicta in culex are almost identical with those of H. praecox given in the plates.

All the figures, except Fig. 61, are magnified 2500 diameters. Fig. 61 is magnified about 70 diameters, the stomach tissues being flattened and extended by the cover-glass.


Hæmamœba Danilewskii (=Halteridium) from Pigeons, Crows etc.
1
2
3
4
5
melanin granules
6
Sporocytes
Hæmamœba relicta (=Proteosoma) from Sparrows, Larks etc.
8
9
10
11
12
13
Sporocytes
Hæmamœba malariæ, quartan fever of Man.
15
16
17
18
19
20
21
Sporocytes
Hæmamœba vivax: tertian fever of Man.
23
24
25
26
melanin granules
27
28
Sporocytes
Hæmomenas præcox: remittent or æstivo-autumnal fever of Man.
30
31
32
33
34
In human blood.
36
37
38
39
40
melanin granules
41
42
44
46
polar body
48
50
Further development
52
In stomach of Anopheles
spermatozoa
43
45
polar body
47
49
51
Further development
57
Development of Zygotes in the tissues of Anopheles
53
54
55
56



R.Fielding-Ould, del. **LIFE HISTORY OF MALARIAL PARASITES.** A.S.Huth,Lith. London

Later stages of Zygotes in Anopheles: formation of 'blasts' or 'vermiform young'

LIVERPOOL SCHOOL OF TROPICAL MEDICINE—MEMOIR III

REPORT

OF THE

MALARIA EXPEDITION TO NIGERIA

OF THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE AND MEDICAL PARASITOLOGY

BY

H. E. ANNETT, M.B., D.P.H. (VICT.)
DEMONSTRATOR IN TROPICAL PATHOLOGY
J. EVERETT DUTTON, M.B., B.CH. (VICT.)
AND
J. H. ELLIOTT, M.D. (TOR.)

PART I. MALARIAL FEVER, ETC.

WITH ILLUSTRATIONS AND PLANS

AT THE UNIVERSITY PRESS OF LIVERPOOL 1901

TO THE

RIGHT HONOURABLE LORD LISTER, F.R.S., LL.D., D.C.L.,

THIS REPORT IS RESPECTFULLY DEDICATED

BY THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE

ISSUED BY THE COMMITTEE

OF THE

LIVERPOOL SCHOOL OF TROPICAL MEDICINE AND MEDICAL PARASITOLOGY

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The expedition is specially indebted to E. S. JAMES, Esq., and Dr. R. A. SHEKLETON, for their most valuable aid whilst at Bonny; and to W. WATTS, Esq., Agent-General to the Niger Company, during their travels up and down the River Niger.

Due acknowledgement must also be rendered for the help of the local agents of the African Association, Miller Bros. and Co.; Oil Rivers Trading and Exploration Company; and the Niger Company.

DIARY OF THE EXPEDITION'S MOVEMENTS

1900

March 21 Sailed from Liverpool per s.s. 'Olenda' (Captain HAMPSON).

April 11 Arrived at Old Calabar, Southern Nigeria.

May 3 One of the members started on a visit to Okoyong, Adiabo, and Ikoneto—a few miles up the Calabar and Cross Rivers—returned May 7.

„ 14 Two members visited Creek Town, on the Calabar River.

„ 17 Sailed for Bonny per s.s. 'Sokoto' (Captain WYNDHAM).

„ 18 Arrived at Bonny.

June 18 Two members started on a tour of 'the creeks.'
Arrived at Bakana (Slave Trees).

„ 20 Visited native town at Top Bakana.
Arrived at Bugama.

„ 21 Arrived at Degema.

„ 23 Visited Egwanga, up the Opobo River.

„ 24 Walked through the bush to Kwatown, four miles from Egwanga.

„ 25 Arrived at the Consulate, Opobo.

„ 26 Visited native town of Opobo.

„ 28 Returned to Bonny.

July 18 One of the members started for Okrika and Degema.

„ 22 Returned to Bonny.

Aug. 1 One of the members sailed for the Opobo River.

„ 2 Left Opobo and reached Akwete.

„ 5 Visited Obunko and Ohumbele.

„ 6 Reached Azumini.

„ 8 Returned to Akwete.

„ 9 Arrived at Bonny.

„ 10 Dr. ELLIOTT returned to Liverpool per s.s. 'Sokoto.'

„ 11 Drs. ANNETT and DUTTON proceed to Degema.

„ 13 Arrived at Akassa, by steam launch through the creeks.

„ 20 Visited Brass.

„ 21 Started for Agberi, on the River Niger.

„ 22 Arrived at Agberi.

„ 23 Reached Abo.

„ 24 Visited Utshi—reached Asaba.

„ 28 Visited Onitsha.

Sept. 4 Started for Lokoja.

„ 5 Passed Idah.

„ 6 Arrived at Lokoja.

1900

Sept.	13	Returned down stream by Niger Company's steam launch 'Nupe.'
„	14	Arrived at Onitsha.
„	20	Visited Abutshi.
„	27	Sailed for Burutu.
„	28	Arrived at Abo.
„	30	Arrived at Ganagana.
„	31	Arrived at Burutu.
Oct.	8	Sailed for Liverpool per s.s. 'Bornu' (Captain HELE).
„	28	Arrived at Liverpool.

REPORT OF THE MALARIA EXPEDITION TO NIGERIA OF THE LIVERPOOL SCHOOL OF TROPICAL MEDICINE AND MEDICAL PARASITOLOGY

I. PRELIMINARY

I.—*Introduction.* Scientific research and investigation in the subject of the aetiology of malarial fever during the last twenty years of the past century furnished results unequalled in their important bearing, not only in the science of parasitology, but more especially on the imperial question of the colonisation of the notoriously unhealthy parts of tropical and subtropical countries.

In 1880 LAVERAN,[1] in Algiers, first discovered adventitious living organisms in the red blood corpuscles of patients suffering from malarial fever, which were characterised by the possession of a dark pigment, and by their capability of executing amoeboid movements.

In 1889 GOLGI,[2] of Pavia, by studying the 'rosace' forms, was able to differentiate between tertian and quartan forms of fever, and to trace out the processes of maturation and sporulation of the parasites and their relations to the periodicity of these fevers.

In 1894, MANSON[3] originated the idea that the malarial parasite was capable of an existence outside the human body, in which the 'flagellate' form played an important rôle. He was led to suggest that the mosquito served as a host for the further development of the parasite.

Ross, in 1897[4] and succeeding years,[5] established the truth of these ideas, and first succeeded in the cultivation of the crescent form of the aestivo-autumnal parasite (*Haemomenas praecox*) in the stomach of *Anopheles rossii*. In 1898 the life-history of a similar parasite of birds (*Proteosoma grassii*, *Haemamoeba relicta*) was worked out by him—from the formation of zygotes from the fertilised female parasite (*macrogamete*) in the stomach wall of *Culex pipiens* to the collection of the germinal rods (blasts, sporozoïtes) in the cells of the salivary glands. The complete biological cycle of the parasite was furnished by the production of infection in healthy birds by the bites of mosquitoes previously fed on infected birds. Confirmation of these

researches, both in general bearing and in detail, has been afforded by the observation of the fertilisation process in the case of halteridum of birds (*Haemamoeba danilewskii*), as well as of human aestivo-autumnal parasites (*Haemomenas praecox*) by MacCallum,[6] and further of the formation of the 'vermicule' in the case of proteosoma of birds (*H. relicta*), by Koch,[7] and in the case of human malaria by Grassi,[8] who also observed by histological processes the passage of the 'vermicule' through the epithelial lining of the stomach of the mosquito.

The Italian pathologists and zoologists, Bastianelli and Bignami,[9] Celli[10] and Grassi,[11] and others having peculiar facilities and opportunities for research in the subject in Italy, have contributed many confirmatory articles, and also by considerable experimental work have brought forward evidence which exculpates mosquitoes of the genus *Culex* from any part in the transmission of human malarial fever.

The interesting experiment of Manson and Thorburn,[12] who, in London last year, allowed themselves to be bitten by mosquitoes infected from a case of tertian fever in Italy, and after fourteen days acquired a typical attack, exhibiting tertian parasites in the blood, afforded a strikingly conclusive confirmation of the work of Ross and of Italian observers.

These discoveries naturally opened up the question as to how the results obtained might be turned to practical account for the prevention of malarial fever, so that commissions and expeditions were despatched to the tropics to ascertain at what period of the life-history of the parasite in man and in the mosquito the parasite is the most vulnerable, and how the attack might best be delivered.

In 1899 there were sent out a German Commission under Professor Koch in German East Africa, a Royal Society's Commission to British Central Africa, and the expedition to West Africa from the Liverpool School of Tropical Medicine under Major Ross. In 1900, another German Commission, under Professor Koch, was despatched to the East Indies; the Royal Society's Commission visited West Africa, and a second expedition from the Liverpool School of Tropical Medicine was sent to West Africa.

Objects of the Expedition.—The experience of Major Ross in India, and the consideration of the results of his work, led to the conclusion that the parasite could be most easily attacked during its life in the mosquito, and as there was considerable evidence to exclude mosquitoes of the genus *Culex* from taking any part in the transmission of malarial fever, the first expedition of the Liverpool School of Tropical Medicine, of which one of us was a member, was undertaken to study the bionomics of mosquitoes of the genus *Anopheles*, with a view to suggesting better modes of prevention of malarial fever than those hitherto known to us.[13] As to the conclusions arrived at and the methods suggested, these will be found fully described in the report of the expedition.[14]

The objects of the present expedition were as follows :—

(1) To further explore West Africa to ascertain under what varied conditions mosquitoes of the genus *Anopheles* lived and propagated, with a view of ascertaining the most feasible and practical methods of preventing malarial fever.

(2) To investigate the conditions under which malarial fever is conveyed to Europeans.

(3) To corroborate and extend recent discoveries and researches on the subject.

It was not intended to limit observations to malarial fever alone, but to study also other tropical diseases as opportunity arose, and to note in addition the general sanitary condition of the places visited.

History of the Expedition.—Nigeria, Northern and Southern, were chosen for the field of operations.

The expedition consisted of :—

H. E. ANNETT, M.D., D.P.H. (Vict.), Demonstrator in Tropical Pathology, Liverpool School of Tropical Medicine.

J. EVERETT DUTTON, M.B., B.Ch. (Vict.)

J. H. ELLIOTT, M.D. (Toronto).

The proceedings of the members of the expedition during the seven months in West Africa are given in the diary of the expedition's movements. The first five weeks were spent at Old Calabar, but the work of the expedition here was almost fruitless, because of the condition of the natives, who are a dull, unintelligent people, understanding almost no English. For the next three months, Bonny, a very old and well-known trading station, was made headquarters, and here most of the work of the expedition was done. Accommodation was spacious, and intercourse with the natives was easy ; English is spoken, and civilisation fairly advanced. Numerous experiments and researches were carried on here, and in view of what the later experience of the expedition proved, it was unfortunate that a longer stay was not made. From the time of leaving Bonny, the work of the expedition became more or less exploratory only. The Niger district of Southern Nigeria having only recently been taken over by the Government, the expedition found at all the places on the Niger and its delta which were visited, accommodation so scanty as to render scientific investigation impossible, in fact, in some places actual living was rough and uncomfortable. In Northern Nigeria, Lokoja alone was visited, and here, again, the accommodation which was available rendered a longer stay than a few days impossible.

II. TOPOGRAPHY AND STATISTICS

Roughly, Nigeria may be divided in its topographical characters into three regions—the region of mangrove swamp, with but little vegetation beyond the mangrove tree, lining the coast and reaching inland to an extent varying from a few to a hundred miles; studded here and there with the low-lying, dirty, swampy towns and villages of the African negroes. Beyond this is the thickly-forested belt—the palm oil region—with much cleaner and often well-arranged towns; and natives engaged chiefly in agricultural pursuits. Further inland is the deforested tract extending to the edges of the central desert. This country is hilly and undulating, and covered with a short scrub and but few trees, and is chiefly occupied by tribes professing Mohammedism. Here and there the depressing monotony of the extent of mangrove swamp is relieved along the course of the rivers or the streams forming the delta, by the occurrence of patches of thickly forested land of small area—anticipating the character of the forested belt further inland.

Old Calabar, the chief town of Southern Nigeria and the seat of government administration, is situated about fifty miles up the Old Calabar river, on its left bank. The river up to here is lined with mangrove swamps and studded with islands of mangroves. The town is placed on the edge of the forested plateau which lies beyond the region of swamp. The mangrove region extends on the opposite bank still some miles further inland. The plateau is about two hundred feet high, where it here comes to the river; it terminates rather abruptly, and slopes rapidly to the water's edge. Old Calabar is built on this slope. Two short spurs jut out, so that the town is surrounded in a semi-circular fashion by the edge of the plateau. On the western half of the slope is the native town of Duketown, which is continued over the summit of the spur into the small village of Henshawtown. A creek, some swampy district, and a small valley separate this native half from what may be called the European half of the town. The European quarters are built mostly on the edge of the eastern half of the plateau; government offices and other administrative premises are dotted about on the slope below. Over the easterly spur is the small village of Oldtown, and Quatown is another small village about one-half to one mile inland on the level plateau. The factories of the various trading companies are dotted about on the water's edge from the extreme end of the native town to the end of the European half.

Proceeding up the hill on the European side of the creek from the Queen's beach and the offices of the marine department, are met with, first the customs and post-offices, and the public works department offices about half way up the hill;

THE NATIVE TOWN OF DUKETOWN—OLD CALABAR

ON THE EXTREME RIGHT AND LEFT ARE TWO EUROPEAN FACTORIES, ALMOST SURROUNDED BY NATIVE HUTS. TO THE RIGHT OF THE FACTORY, JUST SHOWING ON THE EXTREME LEFT, IS THE 'CREEK.'

then nearer the top is the Vice-consulate, and on the summit are the Consulate and Medical house. Further round are the Force house and barracks of the West African Frontier Force, the prison, the European hospital, and a Presbyterian mission station.

On the edge of the creek also on the European side of it are built the native hospital and the dwellings of native clerks, court messengers, and orderlies. It is not permitted that native houses should be built on this side of the creek, thus procuring a distance of about half a mile between the government European quarters and the native town. The few native huts which have existed there for a long time before the present European quarters were built are being gradually removed.

The vicinity of these quarters has been excellently laid out, and is kept in remarkably good order. Excellent roads, well ditched with cemented gutters, cross the hill in all directions ; the quarters are fairly well designed. The total European community at Old Calabar numbers about one hundred and twenty.

Duketown presents a marked contrast to the European half of the town. Here the native huts are crowded together, although some efforts have been made by the medical and public works officers of the district to construct wide roads and to improve the sanitary condition of the town. Narrow winding and irregularly made paths run down the hill side between the native huts which, reaching down to the beach, are crowded round the factories of the trading companies there. On the whole, however, the huts, which are of clay, are fairly clean, although the Calabar native is unintelligent, lazy, and but little influenced as yet by civilisation. Another mission station is built near the summit of this hill, surrounded by native houses.

Throughout the native town and villages on the river's edge numerous dug-out canoes, drawn up to the neighbourhood of the native huts, formed the principal breeding-places of *Anopheles*—and here and there a 'puddle' in the clay at the sides of the paths contained larvae. On the edges of the 'creek,' besides innumerable canoes, several small puddles in this swampy district were found to contain larvae also.

A narrow footpath runs along the whole length of the beach behind the trading factories, and from one factory to the next. This is badly constructed, and permits of the formation of shallow pools during the rains—we observed these several times with innumerable *Anopheles* larvae. The proximity of the native huts to the factories, and the presence of many breeding-places of *Anopheles* in their immediate vicinity, explain the prevalence of malarial fever among the Europeans here, who, moreover, from the low position of the factories on the water's edge and from the general contour of the district, are denied the enjoyment of those refreshing breezes which is secured by the Europeans in the more favourable position on the hill.

Near the quarters of the Government officials on the hill no breeding-places of *Anopheles* could be found during the period of our visit, except a small 'duck'

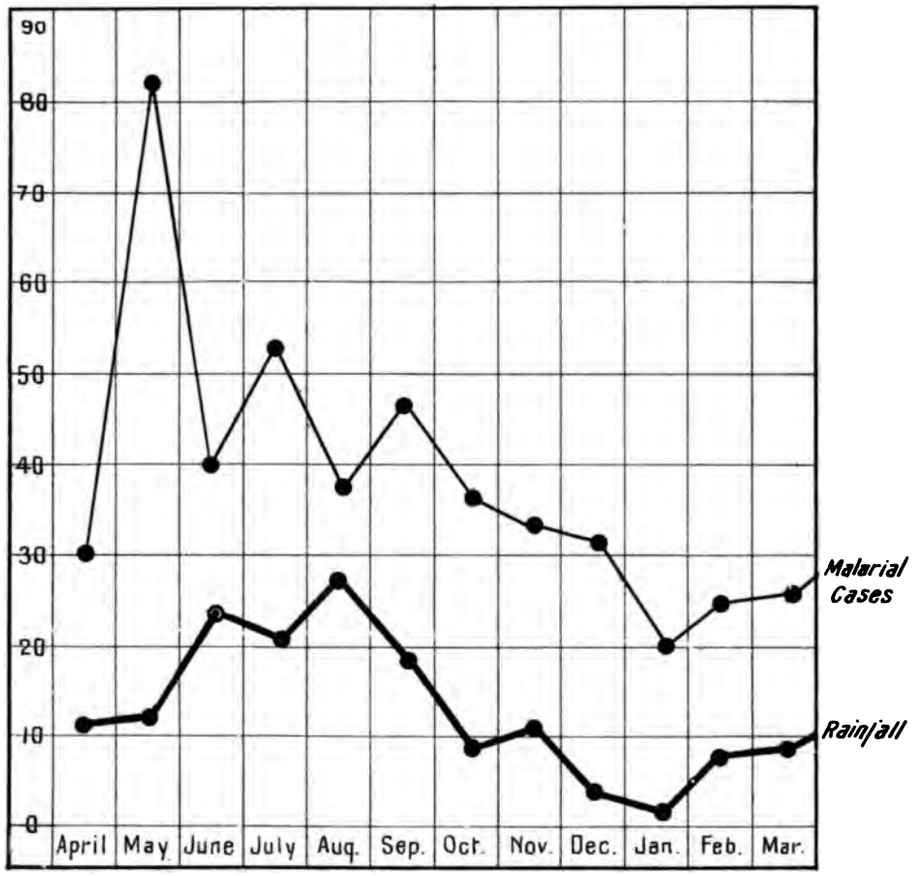
pond near the consulate. The water of this cemented pond was so often disturbed by fowls, turkeys, and other poultry, that never more than very few larvae were found here. Since our visit it has been regularly emptied.

The water supply of the natives is a spring in the valley above the 'creek'—that of Europeans is rainwater caught from the roofs of their quarters, and stored in large iron tanks. A scheme is on foot, however, to bring water to the town from a spring about two miles distant, in the bush, and engineering operations are at present being carried out. The spring occurs at the bottom of what appears to be a natural depression some one hundred feet deep. A well has been already bricked out here to the depth of some eight feet, and the neighbourhood of this was at the time of our visit flooded over a small area around. Here innumerable larvae were found. The district is visited by many natives for drinking water, and a number of labourers are employed during the day in the structural operations. These and the occupants of a small hut on the edge of the depression are the only people within half a mile of the place. The presence of such a large number of *Anopheles* larvae was therefore remarkable.

Of the other places in Southern Nigeria visited by the members of the expedition but little description need be here given—they will be sufficiently referred to in subsequent chapters. The European communities at these places seldom exceed ten in number.

Akassa must, however, be particularly referred to as a place which, in our opinion, should be at once abandoned by Europeans. A vice-consulate and the engineers' quarters are the only European habitations here, and these have been built on 'made' sites in the midst of the mangrove swamp. Here are the only engineering yards of the country, which were taken over from the Niger Company by the Government at the beginning of last year. Were not the swampy nature of the district sufficient reason for abandoning the place, it has been rendered almost uninhabitable because of the prevalence of malarial fever among the Europeans—brought about by the presence of the small native village on one side and of the dwellings of the native artisans and labourers with their families on the other side of the engineering quarters, while the proximity of the barracks of the native soldiers is a perpetual menace to the health of the officials at the vice-consulate. To abandon this place and to transfer administrative quarters and engineering yards to Brass, a comparatively healthy district some twenty-five miles off, where already an excellent vice-consulate building exists, would, in our opinion, involve the expenditure of a much less sum than will be required to make Akassa inhabitable and at all healthy.

Statistics.—It is very desirable that fuller and more detailed accounts of the meteorological conditions occurring at the various stations of Nigeria should be kept: at present there is a deplorable dearth of instruments for these purposes throughout the colony. It is only with considerable difficulty that we have been able to gather



It is to be noted how closely the number of Cases of Malarial fever among Europeans, follow the variations in the rainfall, and especially how the largest number of cases occur at the time when the rainy season has become fairly established.

Lokoja is the most important town of Northern Nigeria. It is situated at the confluence of the Niger and Benue rivers, at a distance of about four hundred miles from the sea coast. Behind the town is a hill—Mount Patti—reaching about one thousand feet in height, and running almost parallel to the direction of the river bank. The hill slopes steeply down for a distance of about seven hundred feet, from which point the slope is less steep and takes the form of four low ridges with intervening vales running at right angles to the river bank. The most southerly of these ridges is called the 'hospital hill'—on which are built the European hospital, the quarters of the medical officers and of nurses and dressers.

The next is the 'barracks' hill with the bungalows of the officers of the First West African Frontier Force. The third may be called the 'Residency' hill, having the quarters of the government resident, the post office, and behind these the houses of a number of native clerks and other government employés. On the fourth hill and on the area beyond between Mount Patti, which here approaches nearer to the river, and the bank is the native town.

A road about three miles in length starts from the neighbourhood of the hospital, and, crossing the barracks and residency hills, enters the native town. On each side of this road, as it passes down the side of the hospital hill and up the barracks hill, are grouped the huts of the native soldiers, not very far distant from the bungalows of the Europeans officers. Several other native houses are dotted about near these bungalows.

In each of the valleys between the hills runs one or more small hill streams.

Between the residency and the river, on the sides of the road already mentioned, a bank and other European quarters are in course of construction.

On the water's edge and completely surrounded on one side and at the back with native huts, which reach up almost to the very boundary rail of the compound, is the factory of the Niger Company. Here there are usually some ten or twelve Europeans employed, while at the barracks live often as many as thirty.

Soldier Town is the name given to the district near the river at the base of the ridge known as 'hospital hill,' which is occupied by the greater number of the soldiers of the force with their families.

It is not difficult when one considers the manner in which the dwellings of Europeans are everywhere surrounded by those of natives at Lokoja, to understand how the town has gained for itself a reputation of unhealthiness, and more particularly the barracks of the West African Frontier Force, which might with very little trouble be made quite healthy.

The European quarters have throughout the town been allowed to become encroached upon by the native huts. As already mentioned, the Niger Company's factory has native houses close to its boundary rails : the bungalows of the officers have a collection of soldiers' huts, and a few other scattered huts not very

PORTION OF THE BARRACK SQUARE AT LOKOJA, SHOWING A EUROPEAN OFFICER'S BUNGALOW, AND TWO OR THREE NATIVE HUTS CLOSE BY. TO THE RIGHT IS A STACK OF ANT-PROOF PILES, SIMILAR TO THOSE ON WHICH THE BUNGALOWS ARE BUILT. THEY ARE PARTIALLY OVERGROWN WITH GRASS. THE TRAPS CONTAIN WATER WITH MANY *Anopheles* LARVAE

far distant from them, while behind the residency are the houses of native clerks. In all these cases it would not be difficult, considering the ease with which native huts can be pulled down and re-built, to remove this dangerous condition.

Moreover, the neighbourhood of the European quarters provided many places for the breeding of *Anopheles*. In the barracks square an interesting condition was met with. The bungalows are supported on ant-proof piles, that is, piles provided with a cup to contain water. In these cups when they happened to have water in them were found *Culex* larvae in great numbers. About the centre of the square was a collection of such piles which, not having been moved for some time, were partly overgrown and hidden by long grass. The cups of these piles were found to contain *Anopheles* larvae. This illustrates the peculiar habits of the *Anopheles*, for though in this undisturbed position they were numerous, in similar cups under the bungalows none could be found. The whole surroundings afforded a beautiful example, shewing the source of infection (there were several native huts scattered about close by), and the means by which the infection was easily carried by *Anopheles* bred close at hand, to the Europeans in their bungalows.

A similar example was also provided by the conditions around the factory of the Niger Company. Between the factory boundary and the native huts we discovered a typical *Anopheles* pool containing numerous larvae. The pool was a portion of a very badly constructed drain which ran through the factory's area, which in its course also afforded several other breeding-places.

Besides these, there were many other breeding places of *Anopheles*. In a subsequent chapter it will be pointed out that the construction of roads and footpaths as carried out in many parts of West Africa provides numerous such breeding places. Such occurred on a large scale at Lokoja. The road already mentioned and other roads off this were found by us so badly ditched on each side, that instead of serving to quickly remove water, they permitted of the formation of a long string of puddles on each side, containing numerous *Anopheles* larvae. In some places, in spite of the fact that there is a good natural slope down the hillsides, the bed of the ditch formed a series of steps, water lodging on each. One road in particular is to be mentioned. It runs down the 'hospital' side of the 'barracks' hill. On each side are huts of native soldiers, at the top the officers' bungalows. This road was reconstructed during our visit to Lokoja, and after the work its condition was as bad as before. These samples serve to illustrate how the unhealthy condition of a European settlement is brought about often by the Europeans themselves, by permitting the natives to settle beside them, and further by the unwitting neglect of elementary sanitary and engineering principles.

Parts of the small hill-streams already referred to as running in the shallow valleys also provided *Anopheles* breeding places. These streams become torrents during heavy rain, quickly washing out any larvae; but, during gentle rains, parts of

them, where they swell out into shallow pools with sluggish stream, form excellent breeding-places, and probably in the dry season, when they almost dry up, the pools last remaining perform a similar function. By wading along the course of some of these streams we are able to locate the breeding-places of *Anopheles* which they provided, always at a distance not exceeding two or three hundred yards from human habitations. Beyond this distance above the district of huts and bungalows we found no larvae.

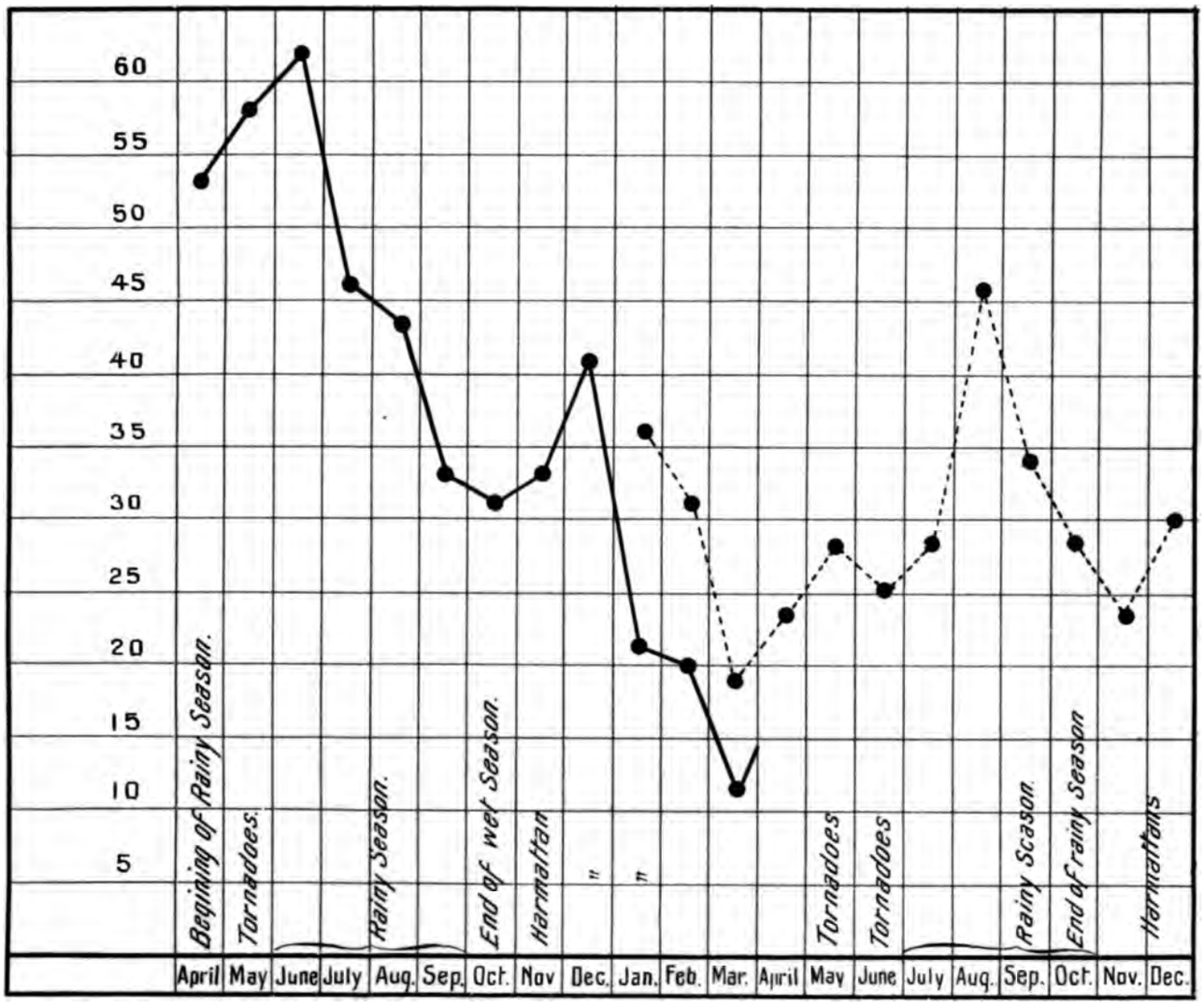
Still another condition was observed at Lokoja. At a point near the river bank we found a small area of cultivated land. We were unable to ascertain precisely the nature of the produce, but it was probably rice. The surface was dug so as to form rows of butts and furrows. One portion of the area was swampy, and water puddles were formed for a short distance along many of the furrows, and also along a narrow footpath crossing the field. In many of these puddles *Anopheles* larvae were found. Habitations in the immediate neighbourhood were scarce, but at a distance of two hundred yards was a collection of native huts.

Statistics.—Northern Nigeria.—The only statistics available are those given in the reports of the medical officers of the West African Frontier Force.

(Chart II)

The points of the line curve represent the percentages per month of the total European force admitted into hospital. Those of the dotted curve shew the actual number of cases admitted per month. Both curves shew a marked increase in the number of cases of malarial fever after the beginning of the rainy season, and the former also a smaller increase after the cessation of the rains. During the course of the wet season itself the breeding-places of *Anopheles* would be continually flooded out, but during its onset and cessation the insects would be able to breed continuously.

The following table has been constructed from the reports of Dr Wordsorth Poole, Principal Medical Officer, W.A.F.F. from January 1898, to March 31st, 1899, and of Dr McDowell, Acting Principal Medical Officer, for the year ending December 31st, 1899.



III. HAEMAMOEBIDAE IN NIGERIA

The Haemamoebidae in Definitive Hosts.—The nature of the expedition and its objects did not permit of a very extensive and minute investigation into the question of the proportion of mosquitoes infected, and the nature of the infection in the numerous places visited. During our three months' stay at Bonny, however, a number of mosquitoes comprising both *Anopheles costalis* and *A. funestus* were dissected and examined microscopically. These were obtained from huts in the native town, as well as from the European quarters of the two factories at Bonny and their outhouses. A total number of two hundred and eighty-one *Anopheles* were dissected, and seven only found to be infected. Roughly, much less than half of these were obtained from the native town, and since, in the light of later observations, it is very probable that the infected insects came from the native town, it can be roughly estimated that, whereas of the total only 2·6 per cent. showed infection, considering the dilution by mosquitoes from the neighbourhood of the factories where no native children dwelt, the percentage of infected mosquitoes in the native town probably exceeded six.

The nature of the infection in the seven *Anopheles* was as follows :—The exact type of parasite—tertian, quartan, or aestivo-autumnal—was difficult to decide.

(1) One mature zygote, showing typical arrangement of zygotoblasts.
(2) One large zygote, showing a few granules of fine pigment.
(3) One mature zygote, with zygotoblasts.
(4) One zygote about half developed.
(5) One zygote about five days old.
(6) Two large zygotes, not quite mature, containing few pigment granules. Salivary glands infected.
(7) Two small zygotes with pigment.

The great rarity with which 'gametes' of any type are met with in the blood of Europeans in West Africa, and the utmost difficulty experienced in work among native children, rendered any experiments requiring the feeding of mosquitoes on infected subjects, which it had been our intention to pursue, impossible during the comparative short stays we were able to make at different stations. Such investigations would require a long stay at one place.

Haemamoebidae in Intermediary Hosts.—The most striking result of the recent scientific expeditions to tropical countries for the study of the aetiology of malarial

fever is the discovery, first by Professor KOCH, the director of the German Malaria Expedition, that the native children of tropical towns and villages are infected to an enormous extent with the parasites of malarial fever, and that they probably constitute the only source from which the disease is conveyed, by the agency of mosquitoes of the genus *Anopheles*, to Europeans. This has been corroborated by the members of the Royal Society's Commission in West Africa, and also by us. Professor KOCH's reports[1] show that he found malarial parasites in the blood of a proportion of the native children under two years of age, to the extent, in some places, of 100 per cent.; of children between two and five, up to 46·1 per cent.; and between five and ten, up to 23·5 per cent. were infected; while in natives over ten, none were found to contain parasites. The proportions obtained by the Royal Society's Commission in West Africa, who also discovered the infection of native children independently, are somewhat higher than these; at Accra[2], from 23 to 90 per cent. of 'babies'; from 20 to 57 per cent. of children up to eight years; from 28 to 30 per cent. up to twelve years; and over that age infection was rare; while at Lagos, of children under two years, from 50 to 100 per cent.; between two and five years, 40 to 75 per cent.; and between five and ten years, 25 per cent. showed either parasites or pigmented leucocytes in their blood, the percentage varying according to the locality.

We were able at almost every town we visited to obtain a number of children for examination. The following tables show our results :—Our method of examination consisted in making a blood smear of about two inches in length and half an inch in breadth. After drying and fixing in absolute alcohol, the preparation was stained by a modification of ROMANOWSKY's method for 'chromatin' staining, and the whole smear carefully examined with a one-twelfth oil immersion objective (ZEISS). The presence of pigmented leucocytes was not specially recorded in the results, the presence of parasites alone serving as the index of infection. The number of parasites found varied from two or three per whole smear up to a large number in every field of the microscope. The ages of the children are given as nearly as could be judged from a general glance. It is possible that in some cases they may be one or two years out. Of those over ten years the majority were from ten to fourteen; only very few between fourteen and eighteen were examined. The different places at which examinations were possible are as follows :—

(*A*) *Bonny Town.*—The native town is close to the Government Vice-consulate and European factories. Many of the specimens were obtained by a house to house visitation indiscriminately, others by visits to huts belonging to one or two Bonny chiefs.

(*B*) *Herbert Jumbo's Plantation.*—This is some five miles up the river from Bonny Town. Here lives the chief, Herbert Jumbo, in the midst of his men and boys and their families, previously 'slaves.'

(*C*) *Ju-Ju Town, Bonny.*—This is the name given to another plantation nearer the mouth of the river, belonging to Chief William Brown. The conditions here are similar to those above described. There are no Europeans in the district.

(*D*) *Akwete Town.*—There are no European traders here, but a Consular Court necessitates the continual presence of one or two Government officials.

(*E*) *Egwanga Town.*—The specimens were taken from children of a small native village, which practically surrounds the traders' factories here.

(*F*) *Onitsha.*—The children who furnished specimens came to the dispensary of the Church of England Missionary Society's station here, or to the school belonging to the same Society.

(*G*) *Akassa.*—Some specimens were obtained from the children of the Hausa soldiers here, who lived in huts in close proximity to the Vice-consulate ; others from children of native engineers, etc., employed in the engineering yard. In the midst of them live three or four Europeans in the Government service.

(*H*) *Asaba.*—The only children obtainable were those of the Hausa soldiers, one or two companies being stationed here.

(*I*) *Lokoja Town.*—The nominal King of Lokoja could prevail upon only very few of his people to provide us with specimens.

(*J*) Others were obtained from the barracks of the Hausa soldiers at Lokoja. The Yoruba soldiers refused absolutely.

	E. Egwanga			F. Onitsha		
Ages	No. Examined	No. Infected	Percentage	No. Examined	No. Infected	Percentage
0-1	3	1	...	0	...	...
1-2	4	3	...	4	2	...
2-3	8	7	...	2	2	...
3-4	3	2	...	5	1	...
4-5	2	1	...	4	1	...
5-6	2	1	...	4	2	...
6-7	2	0	...	5	0	...
7-8	0	...	...	2	1	...
8-9	1	1	...	1	0	...
9-10	0	...	...	2	0	...
10+	0	...	...	0	...	...
0-5	20	14	70·0	15	6	40·0
5-10	5	2	40·0	14	3	31 0
10+	0	...	...	0	...	...
Total...	25	16	64·0	29	9	21·4

	G. Akassa			H. Asaba		
Ages	No. Examined	No. Infected	Percentage.	No. Examined	No. Infected	Percentage
0-1	3	1	...	5	3	...
1-2	2	0	...	1	1	...
2-3	1	1	...	2	2	...
3-4	1	0	...	3	2	...
4-5	0	...	...	2	2	...
5-6	2	2	...	0	...	...
6-7	1	0	...	2	0	...
7-8	2	1	...	1	0	...
8-9	0	...	...	2	0	...
9-10	0	...	...	2	2	...
10+	0	...	...	0	...	...
0-5	7	2	28.5	13	10	76.9
5-10	5	3	60.0	7	2	28.5
10+	0	...	...	0	...	...
Total...	12	5	41·6	20	12	60.0

	I. Lokoja Town			J. Lokoja Soldiers		
Ages	No. Examined	No. Infected	Percentage	No. Examined	No. Infected	Percentage
0-1	0	...	...	7	4	...
1-2	1	1	...	6	6	...
2-3	1	0	...	4	3	...
3-4	0	...	...	1	0	...
4-5	2	1	...	2	1	...
5-6	1	1	...	1	0	...
6-7	0	...	...	0	...	...
7-8	1	1	...	0	...	...
8-9	1	0	...	0	...	...
9-10	2	0	...	0	...	...
10+	7	2	...	0	...	...
0-5	4	2	50·0	20	14	70·0
5-10	5	2	40·0	1	0	...
10+	7	2	28·5	0	...	...
Total...	16	6	37·5	21	14	66·6

These tables show that the number of infected children varies from place to place from 22·9 to 66·6 per cent. ; that over ten years of age very few are infected ; that children between 0 and five years are infected to a great extent.

The following table shows the total numbers and percentage of children infected at different ages throughout the whole country traversed by the expedition.

TABLE SHEWING THE TOTAL NUMBER OF CHILDREN EXAMINED AND INFECTED THROUGHOUT NIGERIA

Ages	No. Examined	No. Infected	Percentage Infected
0-1	37	10	27·3
1-2	46	29	63·0
2-3	49	31	63·0
3-4	47	24	51·0
4-5	41	20	48·8
5-6	23	8	34·8
6-7	30	2	6·6
7-8	25	11	27·5
8-9	16	4	25·0
9-10	14	2	14·2
10+	40	4	10·0
0-5	220	114	51·8
5-10	108	27	25·0
10+	40	4	10·0
Total...	363	145	39·9

By plotting down to scale the figures of the last table, with ages as abscissae, and percentages of children infected as ordinates, a curve is obtained shewing the relation of age to infection. This demonstrates that the number infected is greatest between the ages one and three, and is large also up to five years. The curve shows a very decided fall in the sixth year, after which there is a slight rise, and a rapid fall again during the ninth and tenth years. As to the reasons for the fall in the sixth year, nothing can be given—it may be only accidental.

(Chart III)

The curve also shews how immunity from malarial fever is acquired among the natives. There is besides a certain inherited immunity, since it is well-known that the children of Europeans are severely, and often fatally, attacked by the disease in the tropics, while the native children seem to be but little affected. A temperature of 103° F. was once noted in a child of six months, and occasionally small children in arms were met who evidently were sick and had temperatures—but beyond these, most of the children seem to suffer but little inconvenience, although an examination of their blood at the time might reveal numerous parasites in every field of the microscope. Immunity appears to be more or less completely established by the time the age of ten years is reached, in some cases, however, a longer period appears necessary.

This acquired immunity lasts for a considerable number of years, in many cases a life-time—the period really depending on the extent and frequency of infection during childhood and on individual idiocyncrasy. It has been observed that in all the places visited by us a proportion of the native children were infected, and that the proportion varied and, therefore, the chances of exposure to infection varied. It is possible that there exist in West Africa towns and villages where no malarial fever exists—the natives of such places on entering a malarious district would suffer almost as severely as Europeans. It was only occasionally that we met a native adult complaining of fever, and then only slight inconvenience was occasioned by the attack, which seldom lasted more than twenty-four hours. The returns of the Principal Medical Officer for Southern Nigeria shew a number of adult natives treated, entered as suffering from malarial fever. As microscopical examination was but very little used as a means of diagnosis, it is probable that, although some of these cases may have been true malarial fever, others are, no doubt, fevers of a different nature. In two cases of adults suffering from fever, examined by us, ring forms were found in fair number.

The actual extent and nature of the infection in individual cases at the time of examination are given below.

It will be seen that aestivo-autumnal, quartan, and tertian parasites were found, and, at the time of our visit, the majority of the cases were aestivo-autumnal, while there were also a number of quartan cases, and only a few tertian. Probably the

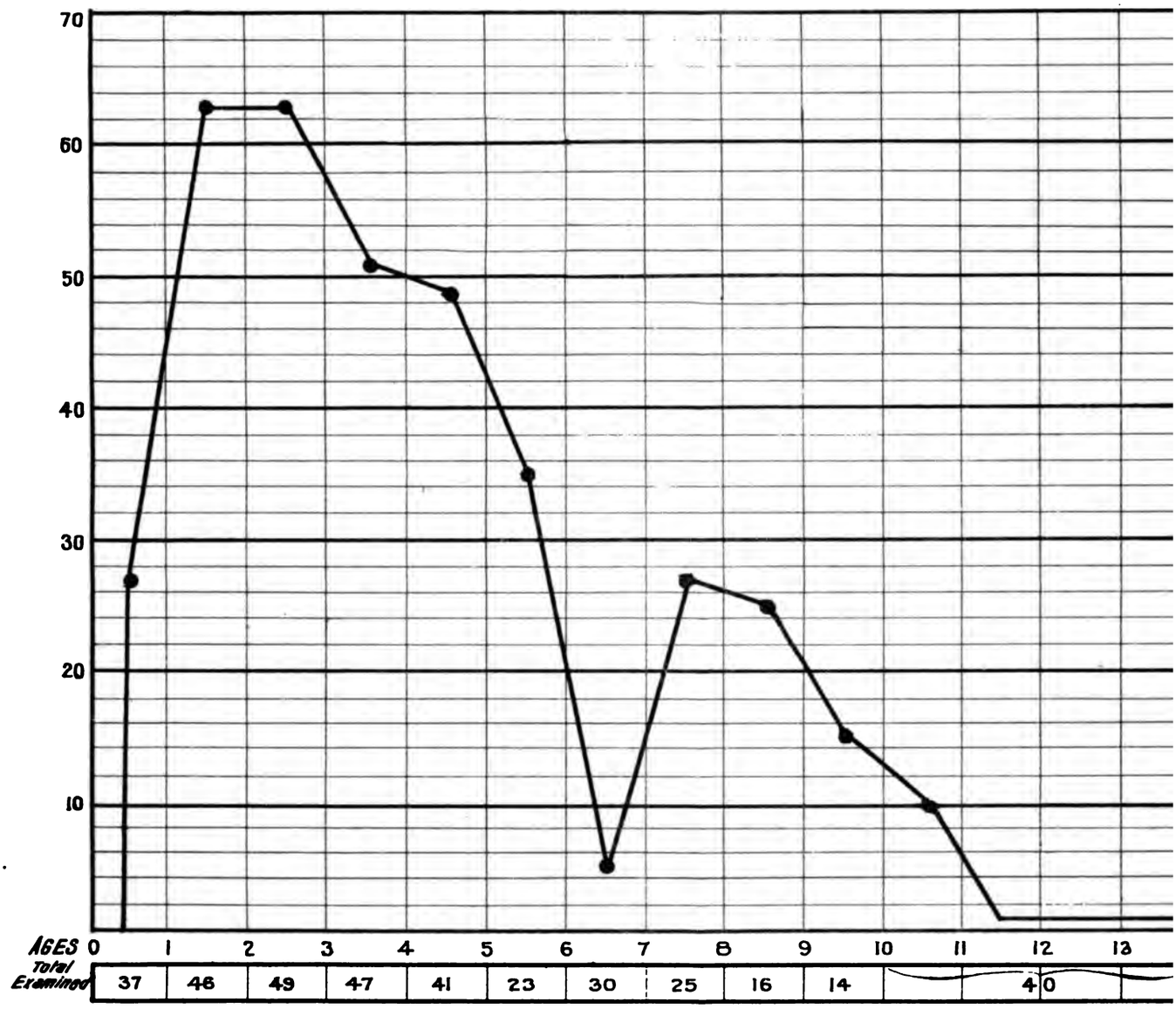


Table shewing relation of age to Percentage of infected Children.

proportional numbers of the cases varying according to the time of the year. It will be noted in the following list that crescents (gametocytes) were frequently met with in native blood, and often several were found in each specimen—they are seldom seen, and then only exceedingly few (one or two per blood smear) in the blood of Europeans in West Africa. In the report[1] of the Royal Society's Commission, it was stated, as the opinion of its members, that quartan and tertian parasites do not exist in West Africa, but our specimens, which exhibit all stages of these parasites, from the young forms to fully matured sporocytes and gametocytes, negative this opinion. It must be pointed out that no crescents were observed by them in the blood of native children.

A. Children of Bonny Town

Age	No. Examined	Result	
0-1	6	All negative	The actual ages, where accurately noted, were three, eight, six, eight and eight months respectively
1-2	6	4 positive	(1) Shewed four crescents (2) An occasional aestivo-autumnal ring form (3) Numerous aestivo-autumnal ring forms (4) One crescent ; one large and two small quartans
2-3	7	3 positive	(1) An occasional ring form ; all stages of quartan parasites (2) Numerous aestivo-autumnal ring forms—all stages (3) " " " " " "
3-4	16	9 positive	(1) Two crescents (2) An occasional aestivo-autumnal ring (3) Seven crescents (4) Several aestivo-autumnal rings (5) Few aestivo-autumnal rings (6) A single crescent (7) A few aestivo-autumnal rings (8) Several aestivo-autumnal rings (9) Numerous aestivo-autumnal rings, mostly of large size
4-5	7	2 positive	(1) An occasional aestivo-autumnal ring (2) Few aestivo-autumnal rings
5-6	3		
6-7	4		
7-8	3	2 positive	(1) Three aestivo-autumnal rings seen (2) Numerous aestivo-autumnal rings—all stages
8-9	2		
9-10	1		
10+	12	1 positive	(1) Numerous aestivo-autumnal rings : actual age twelve-thirteen years

B. Herbert Jumbo's Plantation—Bonny

Age	No. Examined	Result	
0-1	2	Negative	Ages : six months and unknown, respectively
1-2	7	2 positive	(1) Several aestivo-autumnal rings (2) Several small and one large quartan form
2-3	4	2 positive	(1) Occasional ring form (2) Numerous ring forms and all stages of quartan
3-4	6	4 positive	(1) Few aestivo-autumnal rings (2) Occasional aestivo-autumnal rings (3) " " " " (4) Few aestivo-autumnal rings
4-5	6	3 positive	(1) Numerous ring forms and all stages of quartan (2) Numerous tertian parasites (3) Several quartan parasites
5-6	2	1 positive	(1) Numerous aestivo-autumnal rings
6-7	4	1 positive	(1) All stages of quartan parasites
7-8	2	1 positive	
8-9	6	2 positive	(1) Very occasional aestivo-autumnal ring (2) Numerous tertian parasites
9-10	1		
10+	18	1 positive	(1) Several aestivo-autumnal rings : age 10-11 years

C. Juju Town

Age	No. Examined	Result	
0-1	1		
1-2	11	8 positive	(1) All stages of quartan parasites (2) Few aestivo-autumnal ring forms (3) Numerous „ „ „ (4) „ „ „ „ (5) „ „ „ „ (6) An occasional ring form (7) „ „ „ „ and a few quartan forms (8) All stages of quartan
2-3	12	8 positive	(1) Few aestivo-autumnal rings (2) An occasional ring form (3) Several young and a few mature quartan forms (4) Few aestivo-autumnal rings (5) Numerous aestivo-autumnal rings (6) Two crescents (7) Several aestivo-autumnal rings (8) Several young quartan parasites—also sporulating forms
3-4	9	5 positive	(1) A large, a medium sized, and a small tertian parasite (2) Many aestivo-autumnal rings (3) Few „ „ „ (4) „ „ „ „ (5) „ „ „ „
4-5	12	7 positive	(1) All stages of quartan (2) Very numerous aestivo-autumnal ring forms (3) Several ring forms, and a few half-matured quartans (4) Several aestivo-autumnal rings (5) All stages of quartan (6) Nine quartan parasites found—almost mature (7) Few aestivo-autumnal rings
5-6	4		
6-7	10	1 positive	(1) An occasional ring form
7-8	9	4 positive	(1) Few aestivo-autumnal rings (2) Several aestivo-autumnal rings (3) Very occasional ring form (4) „ „ „ and two crescents
8-9	2	1 positive	(1) Several ring forms and a few mature quartans
9-10	1		
10+	1		

D. Akwete Children

Age	No. Examined	Result	
0-1	10	1 positive	The ages given were three, nine, five, four, four, six, six, eight, and seven months, respectively (1) The positive case was eight months old and contained many young quartan forms, also a few mature and sporulating forms
1-2	4	2 positive	(1) Few aestivo-autumnal ring forms (2) „ „ „
2-3	8	3 positive	(1) Many aestivo-autumnal rings (2) Few „ „ „ and two crescents (3) Several „ „ „
3-4	3	1 positive	(1) Few aestivo-autumnal rings
4-5	4	2 positive	(1) „ „ „ „ —all stages (2) Very occasional ring form
5-6	4	1 positive	(1) Several aestivo-autumnal rings
6-7	2		
7-8	5	1 positive	(1) Few aestivo-autumnal rings
8-9	1		
9-10	5		
10+	2		

E. Egwanga Children*

Age	No. Examined	Result	
0-1	3	1 positive	Few aestivo-autumnal rings ; age 6 months ; had a temperature of a 103° F. at time of taking blood ; negative cases, ages 2 months and 5 months
1-2	4	3 positive	(1) Numerous aestivo-autumnal rings (2) Few „ „ „ (3) Many quartan forms—all stages
2-3	8	7 positive	(1) Few aestivo-autumnal rings (2) Numerous aestivo-autumnal rings (3) Very numerous aestivo-autumnal rings (4) Several aestivo-autumnal rings—one crescent (5) Numerous aestivo-autumnal rings (6) Several aestivo-autumnal rings (7) A few crescents
3-4	3	2 positive	(1) Few aestivo-autumnal rings (2) „ „ „ several crescents
4-5	2	1 positive	(1) Few aestivo-autumnal rings
5-6	2	1 positive	(1) Few ring forms, one large quartan parasite
6-7	2		
7-8	0		
8-9	1	1 positive	(1) Very few ring forms
9+	0		

* Since going to press Dr. Hanley, of Opobo, has forwarded more blood smears of native children at Egwanga, which have also been examined.

(1) Age about 6 years—Several ring forms
(2) „ „ 8 „ Negative
(3) „ „ 5 „ Negative
(4) „ „ 5 „ Negative
(5) „ „ 4 „ Many large ring forms
(6) „ „ 4 „ Few ring forms
(7) „ „ 9 „ Two ring forms seen
(8) „ „ 8 „ Numerous quartan parasites—many sporulating
(9) „ „ 5 „ Negative
(10) „ „ 5 „ Many aestivo-autumnal rings
(11) „ „ 10 „ Negative
(12) „ „ 6 „ Numerous aestivo-autumnal rings
(13) „ „ 6 „ Occasional ring form
(14) „ „ 15 „ Negative
(15) „ „ 6 months—Few ring forms, three crescents

F. Onitsha Children

Age	No. Examined	Result	
0-1	0		
1-2	4	2 positive	(1) Several aestivo-autumnal rings (2) Numerous aestivo-autumnal rings
2-3	2	2 positive	(1) Two crescents (2) Few aestivo-autumnal rings
3-4	5	1 positive	(1) Many quartan parasites
4-5	4	1 positive	(1) Few quartan parasites
5-6	4	2 positive	(1) Occasional ring form (2) „ „ „
6-7	5		
7-8	2	1 positive	(1) A single crescent
8-9	1		
9-10	2		
10+	0		

G. Akassa

Age	No. Examined	Result	
0-1	3	1 positive	(1) Several aestivo-autumnal rings (5 months)
1-2	2		
2-3	1	1 positive	(1) Few aestivo-autumnal rings
3-4	1		
4-5	0		
5-6	2	2 positive	(1) Few aestivo-autumnal rings (2) „ „ „ „
6-7	1		
7-8	2	1 positive	(1) Numerous aestivo-autumnal rings

H. Asaba—Soldiers' Children

Age	No. Examined	Result	
0-1	5	3 positive	(1) Very occasional ring form : age 5 months (2) Numerous tertian parasites—all stages (3) Few ring forms, and a few half-matured tertian forms
1-2	1	1 positive	(1) An occasional ring form
2-3	2	2 positive	(1) Numerous aestivo-autumnal ring forms : one crescent (2) Few „ „ „ „
3-4	3	2 positive	(1) Several aestivo-autumnal ring forms (2) Numerous „ „ „ „ two crescents
4-5	2	2 positive	(1) Few „ „ „ „ (2) „ „ „ „ „
5-6	0		
6-7	2		
7-8	1		
8-9	2		
9-10	2	2 positive	(1) Few aestivo-autumnal rings (2) A very occasional ring form

I. Lokoja Town

Age	No. Examined	Result	
0-1	0		
1-2	1	1 positive	An occasional ring form
2-3	1		
3-4	0		
4-5	2	1 positive	Two crescents
5-6	6	1 positive	A single ring form seen
6-7	0		
7-8	1	1 positive	A single crescent
8-9	1		
9-10	2		
10+	7	2 positive	(1) Age 12-13 years; few large quartan forms (2) Occasional ring form: age 10-11 years

J. Lokoja—Barracks

Age	No. Examined	Result	
0-1	7	4 positive	(1) Few aestivo-autumnal rings—one crescent (2) Several „ „ „ (3) Numerous aestivo-autumnal rings (4) An occasional ring form
1-2	6	6 positive	(1) Several aestivo-autumnal rings (2) Numerous „ „ „ (3) An occasional ring form (4) Numerous aestivo-autumnal rings (5) „ „ „ „ (6) „ „ „ „
2-3	4	3 positive	(1) Few „ „ „ (2) Several „ „ „ (3) An occasional ring form
3-4	1		
4-5	2	1 positive	(1) Several aestivo-autumnal rings
5-6	1		

Haemamoebidae in Europeans. The habit of taking quinine, prevalent among Europeans in West Africa, on every occasion of indisposition, makes the examination of their blood, during an attack of malarial fever, very difficult. Occasionally we met with a case which showed numerous parasites in every field of the microscope, but more generally found very few, and often no parasites at all. It is common experience that parasites often rapidly disappear from peripheral blood on the administration of quinine.

Dr. Hanley, the District Medical Officer at Opobo, was kind enough to make specimens of blood smears for us from all the Europeans available in his district, independent of the presence of an attack of 'fever' at the time. The results, with some remarks, are given in the following table :—

Name	Length of present stay on coast	Total time on the coast	Result and Remarks
F	4 months	...	Has had 7 years on the Coast, then 7 years away previous to present visit. No parasites
H	10 months	...	Negative
P	16 months	...	Negative
A	2 years and 4 months	10 years	Negative. Has had but little fever. Haemoglobin estimated at 65 per cent.
R	4 months	...	Negative. Had attack of fever 15 days after arrival on Coast
R (2nd specimen)	4 months	...	Showed a very occasional ring form. Temperature at time of taking specimen, 101° F.
P	5½ months	10 years	Negative. Haemoglobin estimated at 80 per cent.
H	18 months	...	3 ring forms found
T	...	30 years	Has an enlarged spleen reaching midway between the umbilicus and the symphysis pubis. Has had fairly good health. Examination of blood, negative. Haemoglobin estimated at 30 and 20 per cent. on the two occasions
T (2nd specimen)	...	...	
G	...	2nd voyage	Negative
McI	12 months	...	One crescent found. Has had bad health lately. One attack of fever eight months after arrival
T	...	2nd voyage	Negative
B	6 weeks	2nd voyage	Negative
M	8 months	...	Negative
T	6 months	11 years	Negative
M	2 years and 4 months	8 years	Negative
D	4 months	6 years	Negative
M	6 months	...	Negative
H	9 months	...	Negative

Other Haemamoebidae

Haemamoebidae danilewskii was found in a large number of birds, viz. :—

Columba livia	(The common pigeon)
Turtur senegalensis	(The wild dove)
Passer diffusus	
Ceryle rudis	(Black and white kingfisher)
Cypselus affinis	(Humming bird)
Alcedo guentheri	(Blue and red kingfisher)

At Old Calabar a flock of pigeons (common tame pigeon), obtained from the native town, were found infected with *Halteridium* throughout, while at a European factory among pigeons which had been in the country for nine months none were found to be infected. The factory was at some distance from native dwellings, and the pigeons had never mixed with any from the native town. Similarly some pigeons from Bugama, which had been in the country some eighteen months, were not infected—they had no opportunity of contact with pigeons from the native town, which is some two miles distant.

At Lokoja a number of tame pigeons were examined—they were obtained from different sources : some were infected, others not—they had lived in contact some weeks.

Our own collection of pigeons, some of which were infected, were together in a wooden cage for some three or four months, but infection remained limited to those originally infected.

Attempts were made to cultivate *Halteridium* in mosquitoes of both genera, but they failed completely.

Haemamoebidae relicta—Proteosoma was never found, although hundreds of birds of many different kinds were examined.

Other blood parasites—Numerous domestic fowls and a number of small bats were examined, but no blood parasites were found.

IV. SOME POINTS ON THE BIONOMICS OF *ANOPHELES*

Breeding-places.—Throughout such an extensive country as Nigeria, it was natural to expect considerable diversity in the conditions under which mosquitoes of this genus breed. The conditions varied roughly in the three different belts of country already described, and mixed conditions occurred where one belt emerged into the next.

In the region of mangrove swamps the native dug-out canoes are almost entirely the habitat of *Anopheles* larvae and pupae, for instance at Bonny, Okrika, Opobo, Bakana Town.

The canoes containing larvae are generally old and unused, and drawn up on to the foreshores of the rivers or the edges of the creeks into the neighbourhood of the native huts. There are, in places, a considerable number of these, which are now and then augmented by the addition of other canoes during a period of rough 'sass' weather. They are always more or less full of water according to the rainfall, and many have a green (protococcus) growth on their sides, or contain algae.

Only occasionally are 'puddles' containing larvae to be found in these mangrove districts, and it may be mentioned here that quite as often *Anopheles* larvae are found in water in the bottoms of broken gin bottles, in old calabashes, on the tops of barrels, in tubs, and old iron pots.

In the neighbourhood of European dwellings in this district, consisting mainly of those of Government officials and traders, and usually built on made sites, it is found that the breeding places of *Anopheles* have been ignorantly made by the 'white man' himself. They consisted of 'duck ponds,' cemented or tubbed in (larvae are, however, never found when ducks frequent these ponds), shallow wells, with their sides protected by a palm oil puncheon or other barrel, and occasionally uncovered rain barrels. In some places, *e.g.*, round the Consulate at Opobo, the nature of the surface is such as to favour the formation of a small fresh water marsh, in the puddles of which larvae exist.

On the small areas of fertile land, which are here and there interspersed on the river banks in the midst of the mangrove swamps, and on which the 'factories' of the various trading companies are usually built, the breeding places of *Anopheles* consist of shallow puddles, scattered here and there, permitted by the unevenness of the surface and lack of any systematic attempt at surface drainage. These 'puddles' are kept full of water during the wet season, and are frequent along the footpaths crossing the areas, and along the sides of warehouses. In fact, the presence of the enormous number of mosquitoes at the factories at Slave Trees, Bakana, and at

PORTION OF THE FORESHORE AT OLD CALABAR

THE UNUSED DUG-OUT CANOES ARE MORE OR LESS FULL OF WATER CONTAINING *Anopheles* LARVAE
ON THE RIGHT IS THE FACTORY OF A EUROPEAN TRADING FIRM; THE NATIVE HUTS
ARE BUILT CLOSE UP TO ITS WALLS

Bugama, which are completely surrounded by miles of mangrove swamp, can be traced to no other source. At the African Association's factory at Slave Trees, no other breeding places can exist. The small area on which the factory stands is cleared, and surrounded also by a mud swamp covered by tidal water twice a day. Here myriads of *Anopheles* were encountered.

In the region above the mangrove swamp—the forested belt—the natives are more of an agricultural than a fishing race, and consequently the 'dug-out' canoe is considerably less in evidence as a breeding place, except in towns on the river banks. This district is flat, and its surface clayey. The natives build their huts of clay, dug out of pits in the immediate neighbourhood of their huts. These often contain water in which *Anopheles* larvae are easily found. In addition, 'puddles' are also present in hollows either weathered or worn out in the clayey surface. In the large ponds and in the pools in the neighbourhood of springs from which the native obtains drinking water, and which generally contained fish, we were never able to find larvae, except in the small pieces of water locked off at the edges as the level of the water fell. These conditions obtain at Onitsha, Abutshi, Asaba, Abonnema, Degema, and Egwanga.

In the immediate neighbourhood of European dwellings in this district, bad surface drainage, or the proximity of clay pits from which the 'boys' obtain mud for huts, or of wells from which they obtain their drinking water, supply sites for *Anopheles* larvae.

In the region above the forested belt, the country merges into a tract of deforested, more or less open, country, undulating and hilly, sparsely wooded, and covered with a short scrub. Here the breeding places of *Anopheles* consist of the pools, and back eddies, and sluggish corners in the course of the small streams running between the hills, of the badly-made ditches along the sides of roughly constructed roads and footpaths ; and occasionally, in swampy districts, of the furrows between the butts and drills of cultivated fields. Such conditions obtain at Lokoja, in Northern Nigeria.

During the period of the expedition's visit to the river Niger, the rainy season prevailed. This river, which, in the height of the dry season (December to March), is reduced to the condition of a small sinuous canal, only admitting of the passage of launches drawing less than three feet of water, rises during the wet season a height of forty feet, and expands into a great river, reaching in parts a breadth of one to one-and-a-half miles. During the fall of the stream in the dry season it is said that numerous pools are left on the sloping banks, which afford breeding places for innumerable *Anopheles*. In some places, such as Asaba, a most rigorous search, both along the river banks and inland during the wet season, failed to reveal any breeding places for *Anopheles*. Pits from which mud is obtained for hut-building purposes are common ; almost each compound has its clay pit, partially filled with clayey

water, often quickly drying up in the hot sun, occasionally so replenished by rain showers as to be for a period more or less full of water; some, however, deep, and always containing water. They also serve in many places as duck ponds. They were examined time after time but no larvae found, although a few *Anopheles* adults could always be procured in the houses in the neighbourhood. It has been surmised that the chief breeding-places of *Anopheles* in these parts are the pools on the river banks above mentioned, and that during the greater part of the wet season *Anopheles* do not breed copiously. Whether this is so, only careful observation during the dry season can determine.

It is to be remarked that *Anopheles* larvae were never found in the bush or at any great distance from human habitation. The greatest distance observed was under half a mile. Near Old Calabar, at a point some two or three miles in the bush, is a spring, which is intended to be utilised for the water supply of Old Calabar. Engineering operations have already been commenced, and a well bricked in to the depth of some eight feet. The immediate neighbourhood of the well is flooded. Innumerable *Anopheles* larvae were found here. The spring occurs at the bottom of a natural depression in the surface, some eighty or a hundred feet deep, which is surrounded with thick forest growth, and approached only by narrow foot-paths. The only habitations in the neighbourhood are a small hut on the edge of the depression, and another one about half a mile along one of the paths. Two native villages occur at a distance of about a mile. The spring is visited daily by scores of natives for drinking water, and a number of labourers are at present carrying out engineering operations. The occurrence of such an extensive breeding-place away from any large collection of natives is extremely remarkable.

The fact that *Anopheles* larvae are occasionally found in those sites which are generally occupied by *Culex*, namely, broken bottles, calabashes, iron pots, barrels, etc., tends to suggest that in places where the natural breeding-places of *Anopheles* become, either from scarcity of rain or in consequence of artificial destructive means, very scarce, *Anopheles* will make use of any available water which will last sufficiently long for the purpose of laying their eggs. The conditions above mentioned were met with principally in the middle of Bonny native town, where the usual breeding-places, the dug-out canoes, were at some distance, and any puddle which might form on the narrow footpaths and streets was continually disturbed by the trampling of passers-by.

Breeding-places of Culex.—These consisted of sites similar to those already described by many authors as occurring in the immediate proximity of dwelling-houses in the tropics—pots, bottles, tins, cans, calabashes, tubs, barrels, iron vessels, rain tubs, water tanks, pools, puddles, canoes, cocoanut husks, the hollows at the junction of the leaves and stems of the banana tree—and any place where water lodges for a few days. Larvae were found in fire-buckets and other vessels inside houses of Europeans.

THE OLD TOWN SPRING NEAR OLD CALABAR

Anopheles LARVAE IN GREAT NUMBERS WERE FOUND IN THE WATER OF THE BRICKED-OUT WELL, AND OF THE FLOODED AREA IN ITS IMMEDIATE NEIGHBOURHOOD

Ova.—Besides the characteristic manner in which *Anopheles* ova are deposited on the surface of water, they can also be distinguished from *Culex* ova in other ways. The ovum of *A. costalis* is roughly ovoid in shape ; its length is about 0·48 millimetre, its total breadth 0·16 millimetre. The anterior end is the broader ; the superior surface is slightly concave antero-posteriorly ; this surface is broad anteriorly, narrower posteriorly, and constricted in the centre, presenting a shape somewhat resembling the sole of a boot ; it is limited on all sides by a striated border. The inferior surface, that in contact with the water, is convex.

The chitin of the lower surface is beautifully marked with hexagonal figures, which are well seen in an empty egg case. The upper surface has no such markings, but at each end has five very small rounded bosses of transparent chitin. The distinguishing feature of the *Anopheles* ovum, pointed out by Ross,[2] is the presence of two lateral wings of transparent cuticle. These have a width of about 0·02 millimetre, and are about 0·3 millimetre long. They are attached one on either side of the ovum throughout the greater part of its length. This cuticular structure has a wide, oval area of attachment, extending below in a semi-circular manner on to the inferior surface, but limited above by the serrated edge of the upper surface. It is hollowed out on its upper surface. The free outer edge is serrated, the whole surface presenting a milled appearance. Slight maceration of the ovum separates this structure from the egg case.

The larva lies with its head situated at the broader end of the egg case. The top of the anterior end of the egg case is broken off in a spiral manner to allow of the escape of the larva.

It was noted that when mosquitoes of the genus *Culex* are forced to lay their eggs on a small surface, *e.g.*, on water in a narrow test tube, the eggs are arranged in a pattern very similar to that of *Anopheles* ova.

When freshly laid, the eggs are whitish in colour, becoming black in the course of an hour.

Actual countings of the number of ova deposited at one laying gave the following numbers : 138, 145, 233, and 179.

Larvae.—*Anopheles* larvae were occasionally found infected with a parasite (*Brachionus*) which caused them to present a fluffy appearance and hindered their growth, so that they continued in the larval stage for an abnormally long period, and at length died.

The numbers of males and females which hatched out from a number of larvae were counted on several occasions—396 larvae produced 185 males and 211 females.

Habits of Adult Anopheles.—In the preceding paragraph it is seen that the numbers of male and female *Anopheles* which hatch out from a batch of larvae are

approximately equal. Nevertheless, out of a large number of *Anopheles* collected inside European and native quarters but very few males were present; from notes made at the time we counted only 22 males to 293 females, but the proportion is probably only about half that, since many females were taken without note being made of their number.

As to what becomes of *Anopheles* during the day, no exact facts have been observed, and whether the majority remain hidden in the darker parts of habitations, or hide among vegetation, is not absolutely certain. But after sunset, clouds of mosquitoes were often observed flying and hovering in characteristic flocks in the neighbourhood of native huts, about eight feet above the ground. On capturing and examining many of these, all were found to be *Anopheles* males.

In native huts it is never difficult to obtain *Anopheles* females; a large number are always found, for example, in the Kroo boys' huts, while perhaps in the European dwelling-house only an occasional one can be caught. In fact, we found it a good practice in many parts where *Anopheles* were apparently scarce in European quarters, to provide the native boys who acted as our servants with mosquito curtains (they often craved for these); a number could be obtained in this way every morning inside the curtains, which had been badly applied by the 'boys.' Many of the Kroo boy servants make themselves rough curtains of any 'cloth' they can obtain; these invariably provide a supply of *Anopheles*.

It is popularly believed that mosquitoes, and more especially those of the genus *Anopheles*, bite only during the evening and night. It is common experience that many of the genus *Culex* bite during the day time; and, as to *Anopheles*, we have often observed these insects alight on different parts of us, and feed voraciously, in broad daylight. It probably depends very considerably on circumstances. If a feed of blood cannot be obtained at evening or night time when the great majority of these insects feed, then they will bite in the day time. For instance, in offices occupied only during the day time, we were able to find *Anopheles* with distended abdomens. In fact, in the darker parts of the rooms, under tables and desks, behind chairs, etc., *Anopheles* were always to be found; and here they rest until an opportunity of feeding is presented to them, be it day or night; or maybe they are disturbed, fly about, and often attack the intruder.

Considerable evidence has now been accumulated to prove that the distance which is traversed by a mosquito is never very great, and extremely rarely reaches so much as half a mile. The fact that their breeding places are always within a short distance of some dwelling, and have only very rarely been found at a distance even of half a mile, negatives the probability of a long flight. The outbreaks of malarial fever on board ships whose crews had never left the ship, were explained when mosquitoes of the genus *Anopheles* were collected on board ships in malarious districts; and cases occurred which, from their long period of incubation, required as an

explanation that these insects should be carried on board for a considerable time after leaving the malarious district. The experience of the expedition afforded proof of the truth of this surmise. In making the tour of the 'creeks' behind Bonny, on board the s.s. 'Sobo,' the first stopping place was Slave Trees, Bakana. This place is renowned in those parts for the number of its mosquitoes, which, as has been previously mentioned, are almost all of the genus *Anopheles*. The ship anchored about a quarter of a mile from the shore; at night mosquitoes came on board in large numbers. In the cabins and saloons were great numbers of them. One night only was spent here. But for a week afterwards *Anopheles* could be observed in the darker corners of the saloons, and even ten days afterwards an occasional one could be caught, although the ship has been out to sea again in the meantime, in fact, had reached Opobo River. Probably in the smaller and closer quarters of the sailors they would have been found still later.

In the report of the expedition to Sierra Leone, the following observations of the propagation of *Anopheles* occur :—

'We also observed that while naturally-fed gnats invariably laid eggs after two or three days, those which had been bred from the larvae in captivity, and had then been isolated and fed in test tubes, *never* did so, although before being isolated they had long been in company with males. The inference is that fertilisation takes place only after the female has been fed. We noted also that, in a cage where many male and female gnats, both *Culex* and *Anopheles*, were kept together for weeks, eggs were never laid—although the insects were fed, as described, on bananas, and the cage contained water for them to lay their eggs in. It seems, then, that a meal of blood is necessary before fertilisation. Lastly, we observed that previously fed and fertilised insects would lay a second batch of eggs after a second meal of blood, without a second fertilisation; but never laid a second batch of eggs without a second meal of blood. That is, one fertilisation suffices for several batches of eggs, but one meal of blood for only one batch of eggs.'

This is summed up in the following sentence—'Although these gnats (*Culicidae* which feed on men) can live indefinitely on fruit, and perhaps juices of plants, the female requires a meal of blood, both for fertilisation and for the development of her ova. In other words, *the insects need blood for the propagation of their species.*'

Proof of the truth of these inferences is afforded by a series of experiments which we carried out, chiefly at Bonny.

Experiment I—Four male *Anopheles* and five females hatched from pupae were placed together in a cage with a small pool of water and fresh banana: all died in four or five days, having laid no eggs; on dissection, the ovaries were found undeveloped.

Experiment II—Repeated the experiment with thirty to forty mosquitoes of each sex. It was noted that many, both males and females, fed on the banana immediately on introduction. All died in nine or ten days; no ova laid.

Experiment III—A number of male and female *Anopheles* were introduced into a cage with water and banana. The females were all fed on blood *once* only, before introduction ; all died within four days—no ova in water or in insect.

Experiment IV—Repeated this experiment with a large number of males and females. The females fed once on blood, afterwards on banana and water. Some few lived twelve days, all were dead on fourteenth ; no ova found. Ovaries undeveloped ; spermathecae empty.

Experiment V—Introduced into a cage a number of females and males, the females had not been in contact with males before feeding : they were fed only once on blood. Five days afterwards, no eggs having been laid, a further number of males and females were introduced, the females having been in contact with males since birth—and having had one feed of blood. Banana and water were kept freshly supplied daily in the cage. All died within eighteen days from the first, thirteen days from the second introduction ; no eggs having been laid.

Experiment VI—A number of *Culex* males and females were placed in a cage with banana and water ; they had no blood ; but lived for sixteen days—laying no eggs.

Experiment VII—A number of *Culex* males and females introduced with banana and water. The females were fed on blood on introduction and again seven days afterwards. Eggs were laid on the ninth day of the experiment, two days after the last feed of blood.

These experiments were performed in small cages, made of mosquito netting, of size about twelve inches long, twelve inches deep, and six inches wide.

Among mosquitoes of the genus *Culex*, copulation was often observed in the small cages of our experiments, but with *Anopheles* never. It was thought possible that these latter required a longer flight than could be obtained in cages of the dimensions given, so that our next experiments were made with a large cage eight feet long by six deep by four wide, into which were introduced a small iron tank and a soil puddle in an earthenware vessel containing water and a number of peeled bananas which were daily replenished.

Experiment VIII

June 8 Thirteen newly-hatched males and twelve newly-hatched females (*Anopheles*) were introduced into the large cage, the females have been fed on the blood of one of us.

„ 9 Introduced some males caught in native quarters and four newly-hatched, blood-fed females.

„ 10 Introduced eight blood-fed females and a number of males, all newly-hatched out.

„ 13 Re-fed thirteen of the above females, some would not feed, others had died.

June 15 Re-fed nine of the above females; introduced ten more freshly-hatched, blood-fed females and ten males, three of which were caught in the native town; three females were found dead on the water.

„ 17 Re-fed thirteen females; one found dead on the water; showed undeveloped ovaries and empty spermatheca.

„ 19 Re-fed eleven females; introduced three caught males from the native town.

„ 21 Re-fed ten females; only one living male can be seen. First noticed the ovaries beginning to swell as a greyish patch on upper surface of abdomen.

„ 23 Re-fed eight females.

„ 24 Re-fed six females; no males can be seen.

„ 26 Re-fed eight females; one female was accidentally killed while feeding. Ova were found fully developed enclosed in their usual chitinous ridged capsule. No spermatozoa in the spermatheca.

„ 28 Re-fed nine females—all except one shewed distended abdomens

„ 30 Introduced three females caught in native town. Re-fed nine females.

July 2 Re-fed eight females.

„ 5 Re-fed six females; eggs were found on the surface of the water in the small iron tank. All the females were apparently full of eggs except one, presumably the one which had laid the eggs. This mosquito was caught and kept by herself in a small cage.

July 7	Only four females remain in the large cage, two of which would not feed—also two males present.	The isolated female was re-fed on blood.
„ 9	Re-fed four females in large cage.	This female laid more eggs; she was again re-fed. The ova of the 5th had not hatched out.
„ 11	Re-fed four females.	More eggs laid; re-fed with blood; eggs of 5th and 7th had not hatched out.
„ 12	The four females and two males remaining in the large cage were removed and placed in a small cage.	Few more eggs laid. None of the previous eggs hatched.

July 13	One female found dead on the surface of the water with developed ova and empty spermatheca. Others re-fed on blood.	Re-fed on blood ; abdomen swelling.
„ 16	One female dead on water ; contained developed ova. Other two females re-fed.	Laid more eggs. Was re-fed on blood.
„ 18	Re-fed ; no ova laid.	Re-fed.
„ 20	Re-fed ; no ova laid.	Re-fed ; more eggs had been laid. None of the previous eggs had hatched.
„ 21	Added eight males to this cage.	Added four males to this cage.
„ 23	One of the two females had died ; contained ova with transparent cuticle ; empty spermatheca.	Six eggs laid. Re-fed on blood.
„ 25	The remaining female died.	Re-fed. Abdomen again swelling.
„ 27		Few eggs laid. Re-fed on blood.
„ 29		Found struggling on the surface of the water ; had lost one leg ; was killed. Ovaries contained thirty-three developed ova with chitinous covering ; empty spermatheca. None of the eggs laid hatched out.

This experiment was repeated in a small cage entirely.

Experiment IX

June 26 Ten females (*Anopheles*) hatched out from pupae, were fed on blood and introduced with a number of hatched-out males, into a small cage with banana and water.

„ 28 Eight of the females were re-fed on blood ; four males caught in the native town were added.

„ 30 Eight again re-fed ; three more town males introduced ; abdomen beginning to swell.

July 2 Re-fed eight females.

„ 5 Re-fed seven females ; two males found dead on water ; eggs laid.

„ 6 Eggs of yesterday hatched out.

July 7 More eggs laid; re-fed four of the five remaining females, the other would not feed.
" 9 Eggs of seventh hatched out. Re-fed four females; one would not feed.
" 11 Few more eggs laid. Re-fed two females; three would not feed.
" 12 Eggs of yesterday hatched out.
" 13 Re-fed five females; no more eggs laid.
" 16 Re-fed three females; numerous eggs laid; one female dead on the water; two appeared thin.
" 17 Eggs of 16th hatched out.
" 18 Re-fed four females; one would not feed; no ova.
" 20 Re-fed three females; eggs laid.
" 21 Placed more males in cage. Eggs of yesterday hatched.
" 23 Re-fed one female; others escaped.
" 25 The remaining female would not feed; more eggs laid.
" 26 Eggs of yesterday hatched out.
" 27 Remaining female dead on water.

Thus our surmise that a long flight was required for copulation proved false, since the ova in the second of the two experiments just detailed had been fertilised and hatched out. Why fertilisation did not take place in the larger cage is difficult to explain, unless owing to the comparative small number of mosquitoes, both males and females, in so large a space, the opposite sexes seldom met. It is to be noted that in this experiment eggs were laid in water in an iron tank preferably to that in an artificial soil puddle, and further, that although fertilisation did not take place still the ovaries developed apparently normal ova, which, however, did not produce larvae.

It is further to be remarked that, with a regular supply of blood (every other day), eggs are laid within eight days of the birth of the adult female from the pupae; and that they continue to be deposited every second or third day afterwards, if water is available, until the death of the insect.

In these experiments it is seen that females in confinement in cages may live and be fertile for a period of at least seven weeks; from which it may be surmised that under natural conditions they might live much longer.

The following experiment shows that even in the absence of males, the ovaries develop ova.

Experiment X

July 17 Introduced five females hatched from pupae; no males added. The females were fed on blood.

July 19 Re-fed the females.
„ 22 Re-fed the females.
„ 25 Five females re-fed.
„ 27 Re-fed two females.
„ 29 Re-fed two females ; ovaries beginning to swell.
„ 31 Re-fed the two females.
Aug. 2 Re-fed the two females ; swelling of abdomen more pronounced
„ 4 Again re-fed.
„ 7 Re-fed.
„ 9 Re-fed.
„ 11 Would not feed.
„ 13 One had disappeared ; the other would not feed.
„ 15 Re-fed both females ; abdomen very swollen ; no eggs laid.
„ 19 Both had disappeared. (Probably eaten by ants).

In order to show that blood is necessary for the development of the ovaries and the formation of developed ova, the following experiment was performed. The females were not fed on blood.

Experiment XI

July 8 Ten females introduced into a small cage with a number of males caught in the native town ; no blood was given ; banana and water also placed in cage.
„ 16 Ten females still alive ; the banana was replenished every day.
„ 18 Eight females alive ; no eggs ; one dead female examined showed undeveloped ovaries.
„ 21 More males caught in native town introduced.
„ 25 One female dead on the water ; showed undeveloped ovaries.
„ 27 Only one female left ; no eggs laid.
„ 29 All dead ; no eggs laid.

Thus, without feeding on blood, female *Anopheles* may live at least twenty-two days on vegetable juices alone, but the ovaries remain undeveloped, although many males may be present.

The next experiment indicates that a regular and frequent blood feed is necessary—as often as every other day, every fourth day being insufficient—for the development of ova.

Experiment XII

July 18 Ten female *Anopheles*, bred from pupae, introduced into a small cage with six males caught in native town.
„ 20 Two females had died.

July 21 More caught males were added.
„ 23 Re-fed five females ; one would not feed ; others had died.
„ 27 Re-fed three females ; two dead on the water showed undeveloped ovaries, and empty spermathicae. Added five more males from native town.
„ 29 All had died.

The following three experiments were undertaken to ascertain whether males from a certain batch of eggs from one adult female could fertilise the females of the same batch ; Experiments XIII and XIV seem to contradict one another in their results. They also serve to show that the males directly after hatching reach sexual maturity very quickly, and are able to fertilise.

Experiment XIII

July 1 Nine females developed from pupae were fed on blood of one of us and introduced into a small cage together with banana and water.
„ 3 Nine females re-fed.
„ 5 Re-fed eight females.
„ 7 Re-fed six females ; one dead on water ; abdomens swelling.
„ 9 Re-fed five females ; one would not feed ; no eggs laid.
„ 11 Re-fed six females ; one egg found on water.
„ 13 Re-fed five. One would not feed.
„ 15 Re-fed five. Few eggs laid irregularly on water ; one female dead on water shewed fully developed ova with chitinous covering.
„ 18 Re-fed two females ; three would not feed ; single egg of 11th had disappeared ; those of 15th had not hatched out.
„ 20 Re-fed four females ; one would not feed ; more eggs laid ; those of 15th had not hatched.
„ 23 Re-fed three females ; one dead ; more eggs laid.
„ 25 Females would not feed.
„ 27 Re-fed three females ; no males present ; more eggs laid.
„ 29 Females would not feed.
„ 31 Two females dead ; one only fed ; no more eggs. Introduced six freshly-hatched males into cage.
Aug. 2 Some eggs on water ; pregnant female dead—ovaries contained eggs with chitinous covering. Eggs of 27th not hatched.
„ 4 Eggs of 2nd not hatched.

Experiment XIV

Aug. 7 Placed in small cage twelve females fed on blood, with a number of males hatched from the same batch of eggs of an adult female *Anopheles*.
„ 9 Re-fed.
„ 11 Re-fed.
„ 13 Re-fed.
„ 15 Re-fed.
„ 17 Re-fed.
„ 19 Re-fed; eggs laid, but accidentally lost.
„ 21 Re-fed; more eggs laid.
„ 23 Re-fed two of the remaining three females; the other would not feed. The eggs of 21st hatched out.
„ 25 Re-fed two females.
„ 27 Re-fed two females.
„ 29 Re-fed two females.
Sept. 1 One dead; the other would not feed—very pregnant.
„ 3 Re-fed the remaining females.
„ 5 Dead; no eggs laid.

Experiment XV

Aug. 7 Thirteen females fed on blood of one of us, and introduced into cage with a number of males, hatched from a different batch of eggs.
„ 9 Re-fed.
„ 11 Re-fed.
„ 13 Re-fed.
„ 15 Re-fed.
„ 17 Re-fed.
„ 19 One only remaining; re-fed.
„ 21 Re-fed the single female.
„ 23 Eggs laid; the mosquito dead.
„ 24 Eggs of 23rd hatched out.

Thus it does not matter whether the males and females are developed from the eggs of a single female or from different females, fertilisation occurs in both cases.

It was intended to continue these experiments after leaving Bonny, but this proved impossible under the circumstances already referred to.

They prove, however, the following points :—

(1) That a purely vegetative existence is insufficient for the propagation of mosquitoes of the genus *Anopheles*.

(2) That blood is necessary for the development of ova.

(3) That the blood must be available regularly—at least every two days—for the development of ova.

(4) That the power of propagation of the species is acquired in a very short time after the production of the *imago*, and is extremely vigorous during the whole life of the insect if feeds of blood are available.

(5) That one act of fertilisation by the male suffices for a considerable period of ova production.

(6) That even in spite of fertilisation not having occurred, ova develop if regular feeds of blood are procurable, and may be deposited on water.

(7) That unfertilised, fully-developed ova may be carried by the female for a considerable period (four weeks in our experiments).

V. THE PREVENTION OF MALARIAL FEVER

At the commencement of this chapter, which will treat of the methods to be adopted for the prevention of malarial fever, the members of the expedition wish very strongly to indicate that the suggestions given are based on their own experiences and observations, and, as other scientific workers have as a result of their researches in other parts of the world suggested somewhat different lines of procedure with the same object in view, we consider the recommendations hereafter suggested as the most suitable, in fact the only possible, if malarial fever in the country of Nigeria is to be successfully combated. Whether they can be satisfactorily adopted in other parts of West Africa we are not, from personal observation, able to say, but it is noteworthy that the members of the only other malaria expedition which has made a long stay on the Coast—namely, the Royal Society's Commission in the districts of Sierra Leone, Accra, and Lagos, have recommended absolutely the same methods.

With a view as to the possibility of preventing malarial fever, it is to be noted that during the life history of the malarial parasite in the bodies of its two hosts—man and the mosquito (*Anopheles*)—the parasite may be attacked or avoided,

A in its intermediary host—man,

(i) during the incubation period of the disease; and

(ii) during the course of the disease.

B in its definitive host—the mosquito of the genus *Anopheles* by

(i) preventing inoculation, that is, their bites;

(ii) the destruction of the insect in any of its stages of development—as ovum, larva, or adult.

A. The only means of attacking the parasite during its life history in the intermediary host—man—at present known is by the action of quinine, either as a prophylactic during the incubation period of the disease, or as a curative measure during the course of the disease.

Professor Koch, as a result of his researches in the East Indies, has recommended the wholesale administration of quinine to Europeans and natives as the most practical method for the prevention of malarial fever in those parts. However practicable it may appear as a preventive measure in the parts visited by him, it is absolutely impracticable in West Africa. Manson[1] also suggested this as one of a number of methods for adoption throughout West Africa. There are a number of conditions which strongly militate against such a course of procedure. It has been shewn in a previous chapter that a large percentage of native children under ten

years of age, and almost all children under five years, are infected with malarial parasites, often in large numbers, and that their blood frequently contains the parasites in that stage—'gametes'—in which they are naturally fitted for the further development of their life history in the mosquito. It is evident that the mosquito, while serving as the definitive host for the malarial parasite, carries the infecting agent from the native children to other natives and to Europeans. As it is the custom throughout the whole of Nigeria and, indeed, throughout the whole of West Africa, almost universally, for the European to dwell in close proximity to the native, the children constitute continually an eminently dangerous source of malarial fever for the European. It is, therefore, evident that if the method suggested—the universal administration of quinine—be adopted, the native must be also treated—as Professor Koch suggested, and apparently carried out successfully in parts of the German East Indies. It is not sufficient that the European alone should use quinine—and, moreover, it is with difficulty that the majority of Europeans on the Coast can be prevailed upon to use quinine regularly and intelligently.

Professor Koch qualifies the possibility of the adoption of this method by the supposition that the people to be treated are an 'intelligent and obedient community.' Exceedingly few of the natives of West Africa, and especially of Nigeria, can be brought under this description—in fact it can be safely stated that throughout the whole of Nigeria, we never met with a community which could be in anyway classed as 'obedient and intelligent.' The native of Old Calabar—the seat of government in Southern Nigeria—is stupid, unintelligent, and indifferent; those of the Bonny and Opobo districts, who have been longer in contact with Europeans, are just feeling the effects of civilisation, but they still look upon any new procedure on the part of the 'white man' with superstitious distrust and perplexity. The natives of other parts of the delta and of the Niger banks are mostly uncivilised, and often run away at the sight of a European: while there are towns in the interior only occasionally visited by 'white men,' or which are absolutely unopened. It is true that the native chiefs are often intelligent and educated men, but these are exceedingly few. It is extremely doubtful whether in such towns as Sierra Leone, Accra, Cape Coast Castle, and Lagos, where civilisation is fairly advanced, the introduction of any such practice as Professor Koch suggests, is at all possible. It is evidently absolutely impossible for Nigeria, even if the cost and freightage of the immense quantities which would be necessary for the purpose, did not put it completely out of question—for there are numbers of towns of population exceeding 5,000, some even reaching 30,000 and 50,000, and even more.

Among Europeans in West Africa, the usual practice as to the taking of quinine as a prophylactic, is to take five grains every day, or five to ten grains when they feel a little indisposed, 'out of sorts,' or when they think of it. Of the inefficiency of the latter as a preventative measure there can be no doubt, and it is

very doubtful whether, by the cultivation of a habit, the former is of any value. Furthermore, it is even questionable whether, in many cases, a larger dose (fifteen grains) at intervals as suggested, has not deleterious effects when taken when in apparent health. We have observed startling effects in some cases after a single administration to healthy persons of even ten grains.

B. It is by attacking the definitive host that the best results in the prevention of malaria fever have been, up to the present, anticipated.

I. Prevention of Biting by Anopheles

(*a*) *Culicifuges and Fumigation.*—The many substances which have been put forward as culicifuges to be smeared on the exposed parts of the body, or to be used as perfumes for the purpose of preventing the bites of mosquitoes, are not only obnoxious in their use but absolutely useless. Moreover, fumigation of premises can be of no practical value. The 'wily' *Anopheles* deserves its appellation to an extent little expected, and such subterfuges as smearing with kerosene, or the use of lavender and other substances can have but little effect, while fumigation is more likely to expel the European rather than the mosquitoes.

(*b*) *The use of mosquito-proof houses and of mosquito curtains.* Manson[2] advises the extensive use of these conveniences, together with a universal dosing with quinine for a period for prevention of malarial fever in West Afriea. It is not evident, from the report of his suggestions available, whether the use is to be limited to Europeans only, or is to be extended to the natives; if the latter case, then the scheme is evidently impracticable, and if the former it is inefficient.

The mosquito curtain is astonishingly misused by Europeans on the West Coast of Africa. We very rarely met with one who used the curtains in a careful and proper manner. Almost all are so placed as to hang outside the bedposts and reach on to the ground, being either free or weighted. This is an improper way of hanging the curtains, which thus act as a trap for those mosquitoes which have taken shelter during the day-time under the bed—as very commonly happens. The majority of the nets were sometimes so torn as to be of no protective use whatever, others had a few holes. All these were practically useless—the persistent *Anopheles* will discover the smallest hole capable of affording its body admission in the search for blood. It was common to hear considerable surprise expressed at the presence of gorged mosquitoes inside these nets regularly every morning.

Further, it is hardly to be expected that persons who neglect the proper use of the simple mosquito curtain, will attend to the nets at the doors and windows of a mosquito-proof house. In fact, we met with such a house in a district where mosquitoes were very numerous, and found the nets in a condition very similar to that of the mosquito curtains. Apart from the impediment to ventilation which would be produced by the use of nets at the doors and windows of a mosquito-proof

house—and this is a serious consideration in climates such as that of West Africa—the habits of the European in that country expose him repeatedly to the bites of *Anopheles* at times of the day when it would be impossible for him to be inside a mosquito-proof house. The offices of the government and the warehouses and shops of the traders it would be impossible to protect in this way, while innumerable people continually enter and leave—and here many *Anopheles* are often very troublesome. Similarly, work being done for the day, it is a common practice with all the Europeans to sit out after sunset in their verandahs, or in the open, enjoying the cool of the evening—exposed to the attack of mosquitoes, which become active at this time of day.

Destruction of the Insect

(*a*) *Of adults.* This evidently can be executed to a very small extent only, and is not to be considered as of any material help to prevention. The ease with which they can be caught is, however, noteworthy—native boys soon recognised the difference between *Anopheles* and *Culex*, and brought us often as many as fifty or more of the former every day, either in test tubes or even in bottles—large numbers in each.

(*b*) *Of larvae—and the extirpation of breeding places of Anopheles.* This presents a very large field of operations against the mosquito. The extermination of the definitive host will naturally occasion the destruction of the malarial parasite.

The chief result of the previous expedition to West Africa (Sierra Leone) was to establish the fact that breeding-places of *Anopheles* are often easy of destruction or of prevention, and that this procedure would, to a large extent, prevent malarial fever in districts where it was thoroughly carried out. Two courses were suggested.

1 Efficient drainage—and the construction of proper roads—and the filling up of pools and puddles of water to prevent the formation of breeding-places.

2 The use of 'culicicides,' to be added to existing breeding-places regularly and intelligently, to kill larvae present, and to prevent future use of the water for the purpose of breeding.

Although the first method was suggested as the only really efficient measure, the second was offered as a temporary measure until the financial condition of the colony would permit of the expenditure necessary for the carrying out of the first, and also for adoption in those rarer circumstances where the more efficient method could not be applied.

The material changes which have been brought about in the prevalence of malarial fever in some towns in tropical and sub-tropical countries, especially in India and Southern Africa, by the introduction of a good drainage scheme, were previously described as the results of general sanitary improvement, and it was decidedly established, before the part played by mosquitoes in malarial infection was even hinted at, that the adoption of good drainage methods was often immediately followed by a more or less

complete disappearance of malarial fever in the district. Now, of course, such improvement must be ascribed to the destruction of the breeding-places of *Anopheles*.

As has already been shewn in previous chapters of this report, the conditions met with in Nigeria are extremely varied. Districts may be very roughly classified, according to the nature of their more common breeding-places for *Anopheles*, as follows :—

(*a*) Those in which native dug-out canoes, and occasionally shallow surface puddles are usual—for example, at Old Calabar, Bonny, Opobo, Okrika, etc. etc.

(*b*) Those with shallow surface puddles only—the areas of many of the factories of the various trading companies.

(*c*) Those having mud and clay pits, wells and pools, and few surface puddles.—Abonnema, Egwanga, Okoyong, etc.

(*d*) Those with 'made' roads, having ditches at the side and small hill streams—Lokoja.

(*e*) The neighbourhood of fresh-water marsh districts in cultivated and occasionally uncultivated areas.—Lokoja, vice-consulate at Opobo.

(*f*) Towns on the banks of rivers which fall considerably during the dry season.—Agberi, Asaba, and many others.

It must be distinctly understood that these do not include all the breeding-places of *Anopheles*, and we are of opinion that it would be extremely difficult, even in a small district, to indicate all the collections of water which might from time to time serve as breeding-places. For we urge that the destruction of the usual breeding-places of *Anopheles* would be followed by the adoption of any piece of water that might be presented if of sufficient duration, and that at length the various unlimited breeding-places usually frequented by *Culex* would be resorted to. Such a condition, however, would be rendered difficult under the circumstances of modern European domestic life.

In almost all of the classes into which districts have been placed according to the conditions under which *Anopheles* breed, an efficient surface drainage is more or less applicable. Many of the compounds of the trading companies' factories (*b*) are roughly kept, and present irregular unlevelled surfaces permitting the formation of numerous shallow puddles in the rainy season. The careful construction of a few gutters and ditches would obviate the evil, and thus destroy almost the whole of *Anopheles* breeding-places in the immediate vicinity of the factories—which often are the only source of *Anopheles*.

The numerous clay and mud pits of districts (*c*) which occur in almost every native town outside the mangrove swamp region, would be difficult to treat by drainage. From them the natives obtain mud or clay wherewith to build their huts and

but it is essential that it be very efficiently and thoroughly carried out; and that the breeding-places should be intelligently searched for.

Further, there are, no doubt, other conditions to which a drainage system could be applied, which have not been included in these remarks, and perhaps others which have not been encountered by us.

II. Destruction of Larvae by the Use of Culicicides

The general use of 'culicicides' as a really efficient method is impracticable. From observations already made, it is clear that the very varying conditions under which *Anopheles* breed do not permit of the general application of any substance which will destroy larvae or prevent their development—applicable either regularly to the surface, as kerosene, tar, etc., or as a substance, such as lime, as has been suggested, which, once introduced, might render puddle, pool, or other collection of water useless for breeding purposes in the future.

Such a duty—in the carrying out of a general practical measure—as the discovery and treatment of *Anopheles* breeding-places, could not be relegated to a native official or number of officials, even under the supervision of a European sanitary officer. The varying and unlimited conditions under which *Anopheles* do and may breed, require the constant attention of one with some special knowledge and previous experience.

In the report of the previous expedition, the use of 'culicicides' was advocated for extensive trial in Sierra Leone, as a result of the observations of the expedition during their visit. And this serves as a striking example of the variability of the conditions at different seasons of the year, for on the visit of the members of the Royal Society's Commission in the dry season, those conditions for which the treatment was suggested had disappeared, and a new set, producing innumerable breeding-places, had arisen for which, in the opinion of the members of the commission, the operations previously suggested were not applicable.

Under certain rare circumstances, apart from any general consideration, the use of 'culicicides' would have to be resorted to as the only method of treatment.

Kerosene regularly applied appears to be the most reliable of a number of proposed culicicidal re-agents: the cheap ideal substance which will have the effect of rendering pools permanently uninhabitable for the larvae is still undiscovered. From a number of experiments performed by us in West Africa with various substances, we are not able to recommend any such substance with the desired properties; further, we deem lime and gas lime, which have been suggested as a possible ideal re-agent, of no value for the purpose.

Our attention having been directed to a statement in the medical journals[3] that in the country in the neighbourhood of Lake Chad, no mosquitoes occur, and

no fever prevails, led us to try the action of the native potash as a 'culicicide.' Native potash is a substance occurring in large quantities as an efflorescence in the country referred to, and to its action of rendering water, in which the potash is dissolved, distasteful to *Anopheles*, is ascribed the freedom of the district from mosquitoes. From our experiments we are unable to infer any decided culicicidal effects (its action was much inferior to that of lime); we are, therefore, inclined to consider the freedom of the district from mosquitoes, if it be really so, as not due to the presence of this substance.

RECOMMENDATIONS

Above we have discussed at some length the practicability of some of the suggestions which have been advanced, either singly or together, as general methods for the prevention of malarial fever in a European community. We have shewn that in a great measure they are unsuitable for adoption on a large scale in Nigeria—partly on account of unreliability and inefficiency—partly because of the enormous expense. But it must not be understood that we wish to discourage in any way the use of the more reasonable of them as measures of some value under certain circumstances, but they are throughout to be considered very subservient to the measures to be suggested; and though by their employment the chances of infection of malarial fever would be more or less diminished, absolute protection against the disease could never be obtained.

I. Segregation of Europeans at a Distance of about half-a-mile from Natives

The fact that the native children especially constitute a perpetual source of danger to the European, as being naturally the source from which he is infected with the parasites of malarial fever carried from the native by the mosquito of the genus *Anopheles*, suggests very pointedly that the safest and surest plan on the part of the European to guard himself absolutely from any chance of infection would be to live at a distance from the native.

This would have been unnecessary, of course, had the possibility of the suggestion of the use of quinine wholesale among natives been feasible, but the enormous quantity of the drug that would have been required, and the more or less uncivilised condition of West African natives, have been shewn to render the plan impossible.

Similarly, it having been shewn how a total and universal destruction of the insects by any practical means is impossible, and, moreover, since mosquito-proof houses are not at all capable of protecting Europeans from the bites of *Anopheles* at all times of the day and in all the varying circumstances of European life in West Africa—since, indeed, they are impracticable on a large scale—the adoption of the measure of segregation from the native is still more strongly indicated

Segregation of Europeans at a distance from all natives offers itself now as the only measure by which absolute freedom from the disease can be guaranteed, and all the scientific evidence that has been collected respecting the cause of malarial fever and the manner in which infection among Europeans is brought about, markedly

AN *Anopheles* POOL—A PORTION OF A BADLY-CONSTRUCTED DRAIN—AT LOKOJA. THE RAILINGS BOUND THE AREA OF THE NIGER CO.'S FACTORY—THE HUTS OF THE NATIVE TOWN APPROACH ALSO UP TO THE RAILINGS

points to the adoption of segregation principles as the only way in which absolute protection from the disease can be assured.

As has already been pointed out several times, it is almost universally the rule in West Africa to find European houses built round by native quarters, a practice which long experience in India has taught Europeans to carefully avoid. The degree of proximity of the native huts varies in different places. For example, at Old Calabar, many of the factories are almost surrounded, except in front, by native habitations ; similarly at Egwanga, the small native town is built by the side and back of one of the factories, the huts abutting on to the boundary walls. Also at the Niger Company's factory at Lokoja the native houses are very close up to the Company's boundary railings. At other places a small collection of native huts in the vicinity of European quarters serves as a continual source of infection—for example, at Akassa, the barracks of the native soldiers are close to the government vice-consulate, and the small native town near to the quarters of the engineering staff.

It is not essential that the children are of natives of the district, those from other parts of the Coast are equally dangerous. Akassa engineers' quarters may be again mentioned as an example where the engineering artisans, chiefly natives of Lagos, Accra, and Sierra Leone, are housed with their families alongside to the European house. A large proportion of these native children were found by us to contain malarial parasites. Similarly, also, at Asaba, the proximity of the barracks of the Hausa soldiers, who have their wives and children with them, is a dangerous menace to the health of the officers at the Force house.

Examples of the opposite condition of affairs might also be given, for instance, at Old Calabar the Government offices and consulate, vice-consulate, and medical house, are comparatively free from malarial fever, it having been established that the natives shall not build on the European side of the creek separating the two slopes on which the native town and European quarters are built. This creek is at a distance of about half-a-mile from the houses mentioned. The nearer proximity of the barracks of native soldiers to the Force house may possibly account for some cases of fever which occur there.

Further, the factories at Slave Trees, Bakana, and Bugama are at some considerable distance from any natives, who dwell on the opposite side of the river ; the Europeans here enjoy a comparative freedom from fever—although the condition of the surface permits of the production of myriads of *Anopheles* on the spot.

Native servants living in the neighbourhood of Europeans form a source of danger which might in a great measure be prevented. Although they themselves cannot be regarded as an at all common source of infection—indeed only a very rare one—their presence round the European becomes dangerous in two ways. It is the custom to have a number of native servants sleeping in or about the quarters of Europeans.

consist of large warehouses and stores erected at considerable expense. Mission stations also generally present similar undesirable conditions.

In the larger towns, such as Old Calabar, the difficulties become almost unsurmountable. Removal of European business premises is entirely out of the question, but the exigencies of trade and administrative work would permit of the occupation of healthier sites during the evening and night, as has been suggested by the medical and public works officers of that town.

But in all such cases which are difficult to deal with, probably a great deal might be done to remove the natives from their present positions near factories. We do not suggest as feasible a total destruction of such native huts at once, but we see no objection why such control over the neighbouring land should not be gradually acquired, so as to bring about the removal of these native huts, and thus remove a dangerous menace to health. These native huts are of but little worth, and are easy to construct in a very short time. It will become a subject for government policy to enable Europeans to proceed in this direction, involving, perhaps, slight compensation to the natives affected, and it is trusted that, after consideration of all the advantages and the economy which would accrue upon the prevention of malarial fever among a community of Europeans, both engaged in their employ and in trade, the government will in the near future adopt such a policy as will help and render easy the adoption of the principle of segregation.

II. The Surface Drainage of Areas round European Quarters

It has already been pointed out that one way in which infected *Anopheles* may filter through from native childrens' quarters to Europeans at a distance of even half-a-mile from them is by means of the quarters of native servants lodged at different points in the intermediate space.

Yet another way is by means of such collections of water as might serve for breeding places. It is easy to perceive that a number of infected *Anopheles* from childrens' quarters in search of water on which to lay their eggs, might have to choose a collection near to European quarters and from it fly to the nearest European habitation.

Complete drainage of the surface and the destruction of all such collections of water as might serve as breeding-places for *Anopheles* must then necessarily accompany the adoption of the measure of 'segregation,' if the community is to continue completely protected from malarial fever attacks, and it is our opinion that such a combination of the practice of segregation and efficient surface drainage would be followed by complete freedom from the disease among Europeans. The details of the various conditions under which *Anopheles* have been observed to breed in Nigeria

PORTION OF A CULTIVATED AREA AT LOKOJA, SHOWING BUTTS AND FURROWS,
IN THE LATTER OF WHICH *Anopheles* PUDDLES OFTEN OCCUR

have already been given, and it only remains to once again emphasise that an efficiently and intelligently executed system of extirpation is necessary.

Under circumstances where at present 'segregation' is impossible, the destruction of breeding-places of *Anopheles* by drainage and other means already referred to would prevent the multiplication of the insect to some extent, and thus render the chances of infection much less. But it is to be remembered that so long as the mosquito is able to obtain feeds of blood she can live for very long periods, during which she is continually on the watch for a suitable opportunity to deposit her eggs, and that the most unlikely and unusual sites would be utilised for that purpose. This shews the necessity for a thorough destruction of water collections and of the great difficulty of eradicating all, especially in native surroundings.

But the creating and maintaining of breeding-places by such means as the construction of bad roads and footpaths, wells, unnecessarily uncovered, and so on, is inviting disaster. Half-a-mile has been fixed as the distance which should separate European and native quarters from a consideration of the habits of *Anopheles*. There is some considerable evidence for the assertion that these insects do not fly far and that they spend their lives very close to the spot at which they were hatched from larvae. It is extremely rare also that a breeding place for *Anopheles* is found at a greater distance than half-a-mile from the dwellings of man or from spots frequented by man.

Objections raised against the principle of segregation. The only objections which we have become cognisant of, have arisen chiefly among the West African merchants. Excluding any reference to expense, the chief objection has been that such a segregation as that suggested would seriously affect trade. It must be remembered that a distance of half-a-mile only has been fixed. We are totally unable to perceive how in any part of Nigeria such a procedure could in any way affect the trade at any of the factories we have visited. We urge that the principal trade is done, not with natives in the immediate neighbourhood of a factory, but with influential chiefs and middlemen, who generally dwell some distance up the rivers. The small retail trade done with natives living close to factories would be quite uninfluenced if they were removed to a distance of half-a-mile. In fact, some factories are situated at more than half-a-mile from any native town, and do excellent trade—to walk such a distance and carry back their wares is a mere nothing to a native.

The only other objection heard of refers to social conditions. It must have arisen from a misunderstanding of what was suggested, probably from the roughest idea of what 'segregation' might mean,—a wholesale removal of Europeans from among the natives, who were to be regarded as unfit for any intercourse or dealing. Such aggravated ideas are evidently absurd, and it is not clear how the adoption of the principle of 'segregation,' as described above, can in any way influence the present social relations between European and native.

In concluding these recommendations for the prevention of malarial fever, it is very important necessary to note particularly point out in what way they can be adopted in individual cases. Presuming, beforehand, that attention be paid in every case to precautions exercisable by every individual—namely, the careful use of mosquito curtains and of suitable clothing to prevent the bites of mosquitoes, as well as the use of mosquito-proof houses in certain circumstances, and the intelligent and regular administration of quinine, a certain amount of care might be exercised by some individuals who meet unusual circumstances.

We have stated that, for a community of Europeans, segregation and drainage are to be relied upon, while the use of mosquito-proof houses, mosquito curtains, quinine, and other measures are subservient to these, and must applicable be individuals; also that, in a segregated community, the servants must also be properly placed.

For military officers in permanent camp, it is advised that particular care be bestowed on the disposition of the huts of the native soldiers—which should be placed at a distance of half a mile from officers' quarters, and those of soldiers with their families at a still greater distance, if possible. In temporary camp, choice of a site on 'segregation' principles is advised, that is, away from the native huts.

Commissioners and other government officials, as well as travellers and explorers, should avoid sleeping in native huts. It is advisable to pitch tents for themselves and their servants and followers for sleeping purposes away from native towns and villages.

Agents and assistants at trading factories will be careful in the choice of sites for new stations, and will as far as possible re-arrange their compounds so as to place their servants and labourers at as great a distance as the extent of their areas permit and, moreover, to ensure that no collection of surface water on the area can exist.

Similar advice might be given to those Europeans engaged at mission stations.

With those engaged on surveying parties or on the construction of railways, the exercise of the principle of segregation is very easy. It is again the custom at each little station to find Europeans surrounded by natives of all sorts and ages. It is evident that at such places where absolute control over the disposition of all houses, tents, and huts is easy, to so place all such native followers and labourers as not to constitute a danger, presents no difficulty whatever. The construction of railways in West Africa has always been associated with many cases of malarial fever, which were formerly attributed to 'the turning over of the soil.' Now they are easily explained by the formation of numerous breeding-places for *Anopheles* created by turning over the soil in the construction of the railway—thus supplying the carrier of the disease; and the practise of permitting labourers and servants with their families to live close to European quarters—thus supplying the

THE ROAD LEADING INTO THE NATIVE TOWN OF LOKOJA.

ALONG ITS SIDES ARE DITCHES FORMING A STRING OF PUDDLES CONTAINING *Anopheles* LARVAE.
TO THE LEFT, BEYOND THE PORTION TAKEN IN THE PHOTOGRAPH, A BANK AND
OTHER EUROPEAN QUARTERS ARE IN COURSE OF CONSTRUCTION.

source of the infection. Such undertakings ought to continue absolutely free from malarial fever, if the above precautions were practised.

For the prevention of malarial fever among European crews on board ship, the regular administration of quinine, according to the suggestions of Professor KOCH, seems to us very feasible. We have shown how *Anopheles* come on board and remain often a considerable time. There is also a possibility of their becoming infected from the few children which are carried from port to port on the West Coast of Africa. The chief source of their infection is, of course, the children of the native towns opposite which the vessels anchor. Professor KOCH suggests that the administration of quinine be undertaken in the following manner, and there is no reason why this should not be regularly carried out by the ships' doctors, after the outward-bound vessels have reached the coast. Fifteen grains of quinine are to be administered on each of two successive days, preferably in the early morning; then a period of seven days without the drug is allowed, and the administration of fifteen grains again continued daily for two days, and then another seven days' interval, and so on, for the whole voyage.

APPENDIX

AN UNCLASSIFIED WEST AFRICAN FEVER

In the *British Medical Journal* of January 26, 1901, S. W. THOMPSTONE, F.R.C.S. and R. A. BENNETT, M.B., M.R.C.S., district medical officers at Old Calabar, gave a preliminary note on an unclassified type of West African fever—to which they proposed to give the name 'hyperpyrexial fever.'

The cases occurred during the stay of the members of the expedition at Old Calabar, and we were able to watch the course of the disease in both cases.

By the kind permission of Drs. THOMPSTONE and BENNETT we are allowed to reproduce the short account of their cases here—and we are indebted to Dr. SHEKLETON of Bonny for the earlier part of the temperature chart of the second case, which occurred at Bonny. Because of lack of hospital accommodation and the impossibility of affording such constant attention as such a case demanded, this patient was shipped to Old Calabar and treated at the European hospital there.

We now give the remarks of the authors of the communications to the *British Medical Journal.*

'CLINICAL FEATURES OF HYPERPYREXIAL FEVER

This fever is generally ushered in by a slight rise of temperature followed by profuse perspiration and a fall in the temperature to about 99° F. After a period of apyrexia of perhaps twenty-four hours' duration the temperature begins again to rise, slowly at first, but when 105° is passed with alarming rapidity, one degree in ten minutes having been frequently observed, and it may reach 107° on the second day. For fourteen or even for thirty days subsequently there is absolutely no tendency for it to fall. The skin acts either very slightly or not at all, and all antipyretic drugs fail.

The tongue is at first furred on the dorsum and red at the edges and tip; late in the disease it becomes dry and shrivelled. There is no enlargement of the liver or spleen. The urine is of normal character and abundant; the bowels are regular or inclined to looseness. The conjunctivae are injected and the pupils contracted. The mind remains remarkably clear in most cases, except when the temperature is at its highest, but constant symptoms in the early days are great anxiety and restlessness.

With regard to the examination of the blood, no plasmodia nor pigmented leucocytes have ever been discovered, but in two of the later cases it was noticed that the blood tended to coagulate the moment it was exposed to the air, so that it was only with great difficulty that satisfactory films could be obtained.

TYPICAL EXAMPLES OF THE DISEASE

CASE I. This patient had been on the Coast for about five months, and had had no fever until the present attack. During the last month he had lived in a tent at a quarry some

twenty miles up the Akwayafe river, and, feeling ill four days before admission, got into a canoe and found his way down to the mouth of the river, where he was picked up by a launch and brought to the hospital. On admission, his temperature was only 99·6° F., and he was apparently going on satisfactorily when, as in the previous cases, his temperature began slowly to creep up—the beginning of a constant tendency towards hyperpyrexia. He was treated by baths, as will be subsequently described. After his fourth bath he was put into a cold pack, which was changed every hour for four days. The temperature then steadied, and eventually he left for home practically well.

Case II. This patient was sent from an out-station on the fourteenth day of his illness, and was still under treatment when the record was made. So marked was the tendency to hyperpyrexia that on two occasions no fewer than two cold baths were necessary in the twenty-four hours, and it was not until the twenty-second day of the disease that it was possible to substitute the continuous pack for the cold bath. From that time until now the temperature has averaged 102°. The patient lies helpless in bed, occasionally showing some glimmering of intelligence, but for the most part remaining unconscious of his surroundings, taking nourishment well, but passing urine and faeces under him. This condition, as far as we could tell, may continue indefinitely, The temperature shows no tendency to assume alarming proportions, and the heart and lungs are acting well.

Charts

Treatment of the Condition

This may be summed up in one word—baths. The patient is put into a cold bath, and his temperature brought down to 100° or 99°. He sleeps for about an hour, and then feels fairly comfortable, but the temperature at once begins to creep up again, and in eighteen or twenty-four hours it is back at 106°, or at the point at which it is considered advisable to check it. Quinine and other drugs have been systematically tried without influencing the course of the disease ; the coal-tar antipyretics are absolutely useless. Cold sponging and packs are incapable by themselves of reducing the temperature, although in a recent case which recovered it was found that by reducing the temperature by means of the cold bath to 101° or 102° it was possible to keep it there by means of cold packs renewed at hourly intervals.

Progress of the Cases

It has been observed that, if the patient is to recover at all, some change for the better is to be looked for about the end of the third week. The temperature, which up to this time would constantly rise at once after the bath, might remain at 102° or 103° for several hours, and perhaps in the course of the next week two or more days might pass without the necessity for a bath. Convalescence is gradual, and it may be six weeks after the onset of the fever before the temperature finally assumes its normal course. On the other hand, in 50 per cent. of the cases which have come under observation a fatal issue has occurred.'

Several examinations of the blood of both cases were made by Drs. Thompstone and Bennett and by ourselves, but neither malarial parasites nor pigmented leucocytes were observed. Several times on the day of the arrival of the second case at Old Calabar, and on many subsequent occasions, we carefully examined the blood both by fresh and stained specimens.

Dr. SHEKLETON, of Bonny, states that he found the ring forms of aestivo-autumnal fever in the blood of his patient on the first day of the attack, but none subsequently.

On the sixteenth day of the disease (two days after arrival at Old Calabar) an estimation of the number of corpuscles gave 4,384,000 red, and 15,000 white per C.M., by the THOMAS ZEISS apparatus ; and the haemoglobin was estimated at 90 per cent. Three attempts to obtain micro-organismal cultivations from the blood on agar-agar and serum failed. A peculiar tendency of the blood to rapid coagulation was noted. Dr. THOMPSTONE states he has never been able to find malarial parasites in other cases of this disease which have come under his observation. It is to be pointed out that these cases occur during the dry season, at a time when the number of cases of malarial fever is smallest. There is often a history of exposure to the sun's heat, rough conditions of life, and hard work.

The onset, symptoms, and course of the disease are quite atypical of malarial fever : there is absence of rigors, shivering, vomiting, severe headache, etc., etc. ; the liver and spleen are not enlarged nor tender : consciousness is preserved often to the end. The disease may last about a week, or be extended to three or four. There is a continual tendency to hyperpyrexia, only checked by cold baths, by which means the temperature is easily reduced, but it gradually creeps up again directly the cold applications are removed. After a hyperpyrexial stage of one to three weeks' duration, there is always a very long period during which the temperature continues above the normal—a very extended lysis—varying during the day not more than a degree, and falling gradually degree by degree every four or five days.

Drugs, including quinine and phenacetin, seem to have no effect on the course of the disease.

Lung trouble caused the death of the second case on the evening of the day previous to our departure from Old Calabar, and, through the kindness of Dr. THOMPSTONE, we were able to hold a post-mortem examination on the case two hours after death, the details of which we are able to record :—

No rigor mortis : but little wasting : no jaundice : small clean sore over sacrum : no trace of syphilis.

Abdomen—No fluid in peritoneal cavity : serous membrane appeared normal, excepting two small patches, one of old, one of recent perihepatitis, on the upper surface of the right lobe of the liver.

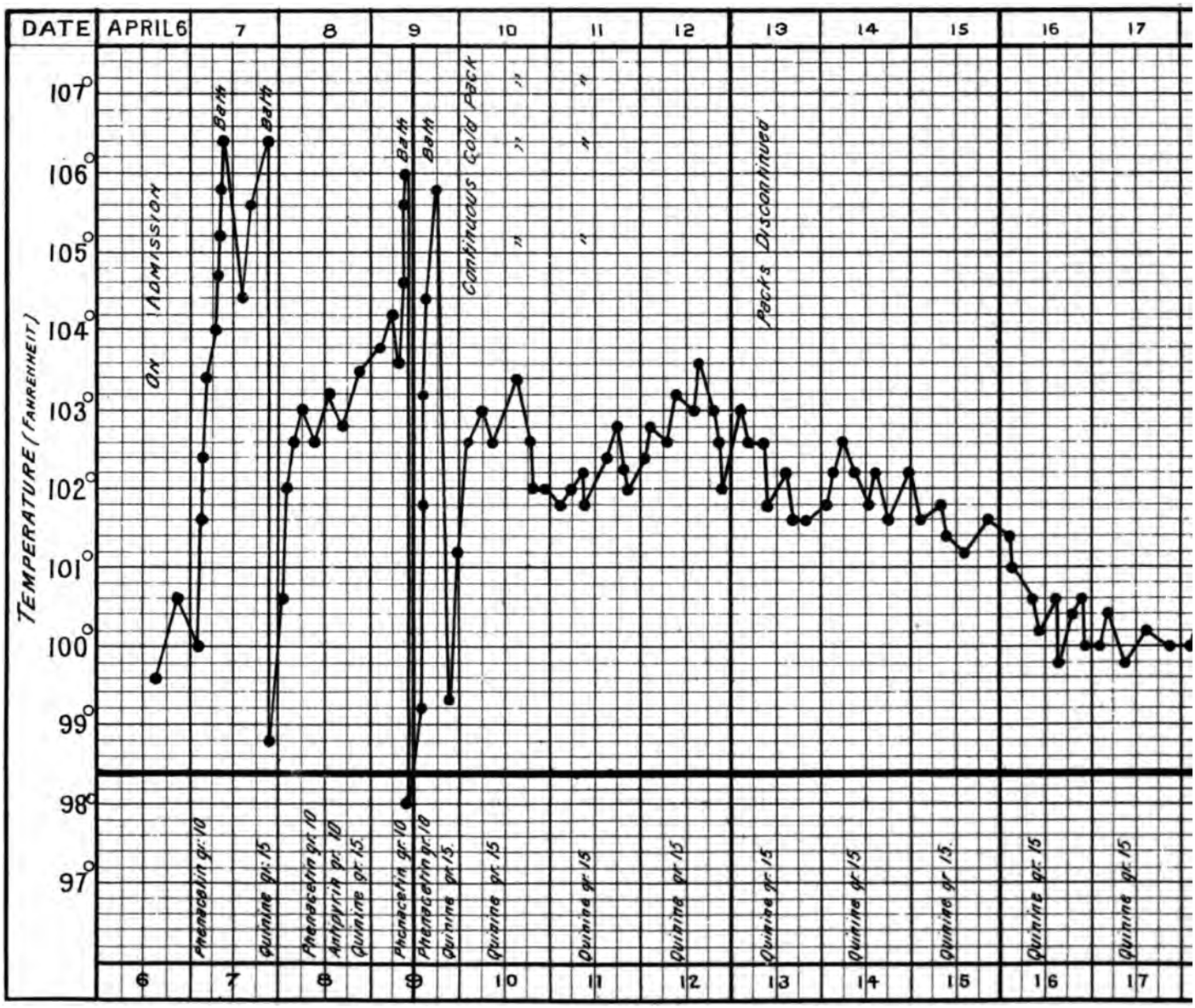
Pancreas—Somewhat large and firm.

Spleen— Did not extend below the costal margin—about the size of one's hand ; firm in texture—externally of slaty appearance ; on section, firm, dark in colour.

Liver—Two small patches of perihepatitis on upper surface of right lobe. Somewhat enlarged and firm ; on section, firm and dark in colour : no sign of fatty degeneration nor cloudy swelling.

Kidneys—Large—capsule stripped uniformly, somewhat adherent all over. On section, red and granular—'large red granular kidney.'

Stomach—Contained a quantity of gruesome, blackish-brown stained mucus in flakes between the rugae. Mucous membrane pale, shewed very small petechial areas to which the blackish shreds were attached.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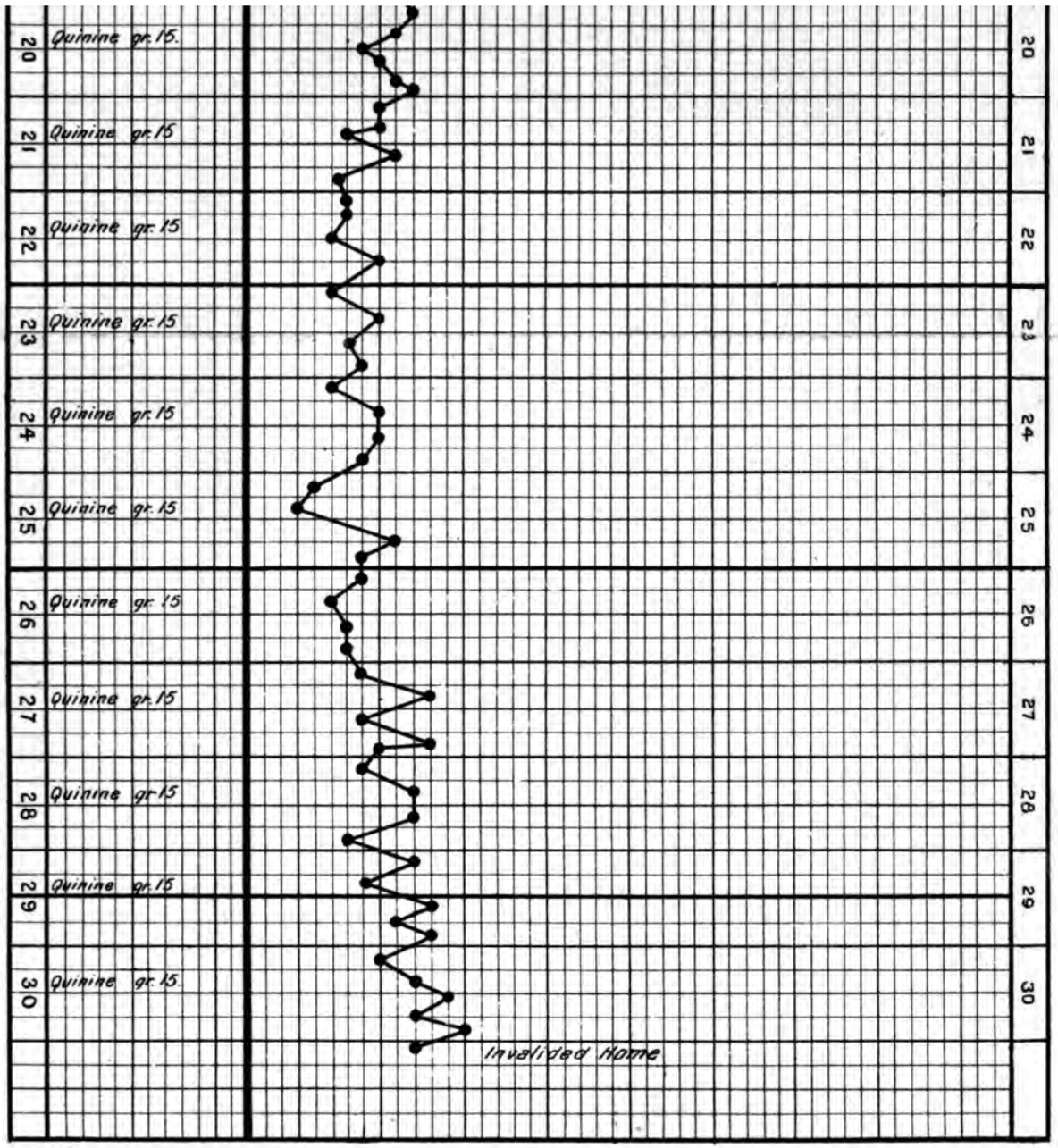
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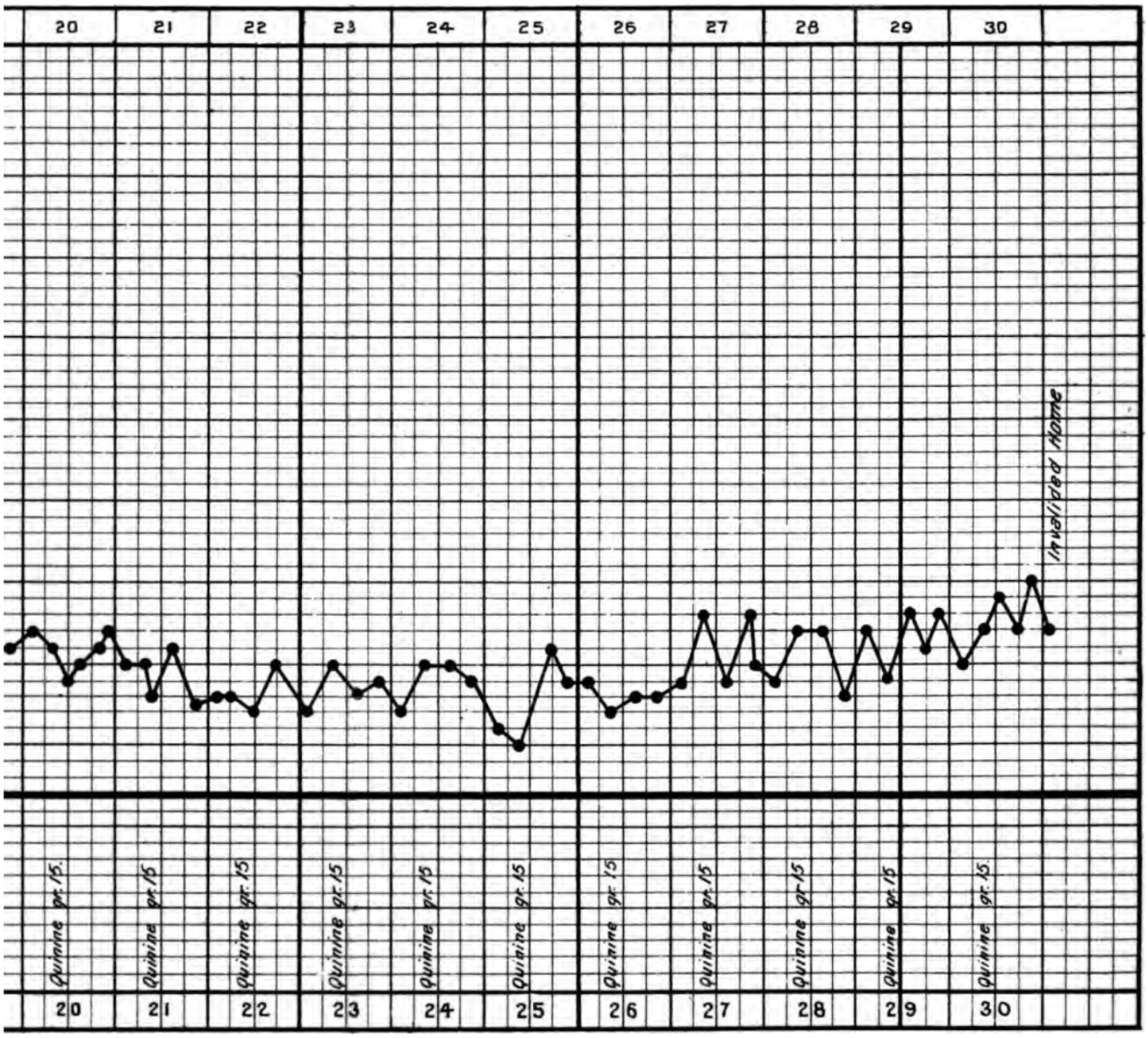
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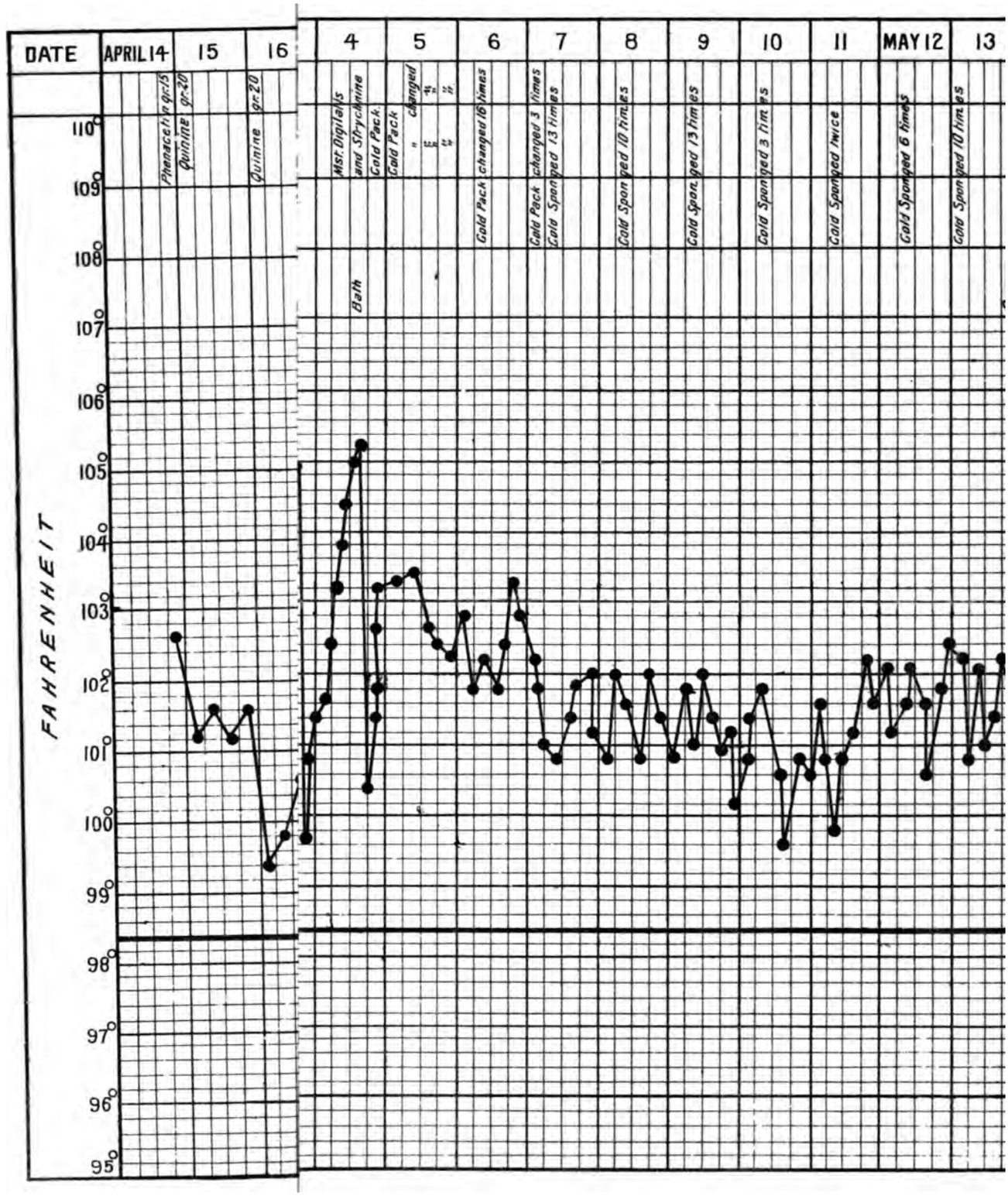
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Cold Sponged 10 times

Duodenum and Small Intestine were stained a dull, greyish-brown colour. About nine inches from duodenum to a few inches from the coecum, the gut containing frothy, blackish-brown, mucous material staining the mucous membrane. No petechial nor haemorrhagic spots. Mucous membrane otherwise normal.

Coecum and large Intestine appeared normal.

Suprarenals and Bladder—normal.

Thorax—No pleuritic fluid—slight old adhesion at the base of the right lung. No periodical fluid.

Heart—Left ventricular wall hypertrophied. Cardiac muscle firm. Valves healthy. Slight early atheroma of the aorta just above the valves.

Lungs—Some old pigmented fibroid patches at both apices. Patch of old pleuritis at right base adhering to the diaphragm. Bronchi contained a quantity of yellowish muco-pus. This condition extended down to the small bronchioles. On section of the lung, muco-pus welled from numerous points. No broncho-pneumonia. No hypostatic congestion.

Oesophagus—Normal.

Trachea—Contained a little muco-pus.

Epiglottis—Two very small ulcers at the base size of pinhead.

Pharynx and Thryroid Gland—Normal.

Brain—Rather wet and oedematous, firm, otherwise appeared normal; numerous small pachionian bodies.

Morbid Histology

Liver—Sections of the liver present very little pathological change. There is a slight increase of fibrous tissue around the portal canals, with a slight thickening of 'Glysson's' capsule. Slight congestion of the hepatic zone. The liver cells are sharply defined—the nucleus staining well with Haematein, the cell protoplasm shews a very slightly granular appearance. Slight fatty infiltration occurs in the portal zones of some of the liver lobules. Marked yellow pigmentary deposit is observed in the liver cells throughout the whole lobule. No recent malarial pigment can be made out in the capillary endothelial cells, and traces of old pigment are only very occasionally seen. The liver cells throughout the lobules give a marked reaction of iron with pure haematoxylin, and with potassium ferrocyanide and hydrochloric acid.

Spleen—Sections shew an increase of fibrous tissue. The trabeculae are thickened and the splenic pulp appears overgrown with fibrous tissue. The small arteries of the Malpighian corpuscles, which are inconspicuous, are greatly thickened both throughout their internal and external coats. The splenic capsule is thickened. Malarial pigment only occurs at very rare intervals throughout the sections. With ferrocyanide of potash and hydrochloric acid, and also with pure haematoxylin no very marked iron reaction is brought out. Masses of haematoidin pigment were scattered throughout the sections.

Kidney—Sections again shew fibroid changes in the thickened capsule of the organ, with a tendency to spread into the kidney substance. Some of Bowman's capsules are thickened—and there is some thickening of the arteries. The renal cells of the tubules are well defined—nuclei stain well—the protoplasm has a slightly granular appearance, and in places there is a slight epithelial desquamation, with a granular deposit in the tubules. There is some congestion of the stellate veins and vessels of the boundary layer. No pigment present.

Lung—Sections shew a condition of marked emphysema. Round some of the bronchi the alveoli are filled with a catarrhal exudation, and a few golden corpuscles are present. The bronchi shew catarrhal inflammatory changes.

Brain —In sections of the cerebral cortex, and of the corpus striatum,* no pathological changes can at present be made out. No malarial pigment is present.

The interesting features to be noted in the results of this examination are the absence of recent malarial pigment from the positions in which it is generally found in cases of malarial fever—and secondly, the absence of cloudy swelling of the parenchymatous epithelium of the organs, and of marked fatty degeneration such as one would naturally have expected as a result of a period of some four weeks' high temperature. It must be stated that the patient had been several years on the West Coast of Africa, and had had several attacks of malarial fever, thus accounting for the presence of a little old pigment.

The absence of cloudy swelling and fatty degeneration changes in the organs points not only to the non-malarial nature of the disease but, we take it, also that a 'toxic' substance produced in the course of a specific infective disease was also absent. It has been suggested that the characteristics of this disease—the peculiar absence of local symptoms, the manner in which the temperature is uninfluenced by drugs, but easily affected by cold applications, and the long period through which it gradually becomes normal, as well as the conditions found on pathological examination—might be all explained by some profound disturbance in the process of heat regulation.

In this connection it may be pointed out, on the West Coast of Africa a temperature of about 130° F. and an atmospheric humidity of between 90 and 100 per cent. are often associated, and that these conditions have considerable influence on the health of Europeans in those parts—accounting for some few of those cases treated as malarial fever cases. From our own observations such cases occur, from which the typical signs and symptoms of malarial fever are absent. It has been noted that no cases of insolation, siriasis, sunstroke, sun or heat fever are found mentioned in the medical reports of Southern Nigeria. It was our experience at Old Calabar that the climatic conditions often produced a slight rise of temperature, and for experimental purposes we occasionally undertook rather severe exercise, such as hard walking (provided with sun helmet or shade) in the open, and almost invariably noted a rise of temperature after an hour or so, although perspiring profusely at the time. Temperatures reaching up to 102·2° F. were observed—normal being reached in the course of a few hours. It is easy to imagine that an exaggeration of such conditions, together with a constitutional condition in which from any cause the dissipation of the heat of the body does not balance the production might, perhaps, lead to such an overthrow of the process of heat regulation as to occasion conditions similar to those of the so-called 'hyperpyrexial' fever cases described.

*Further investigations in the pathological histology of the corpus striatum in this case are being undertaken.

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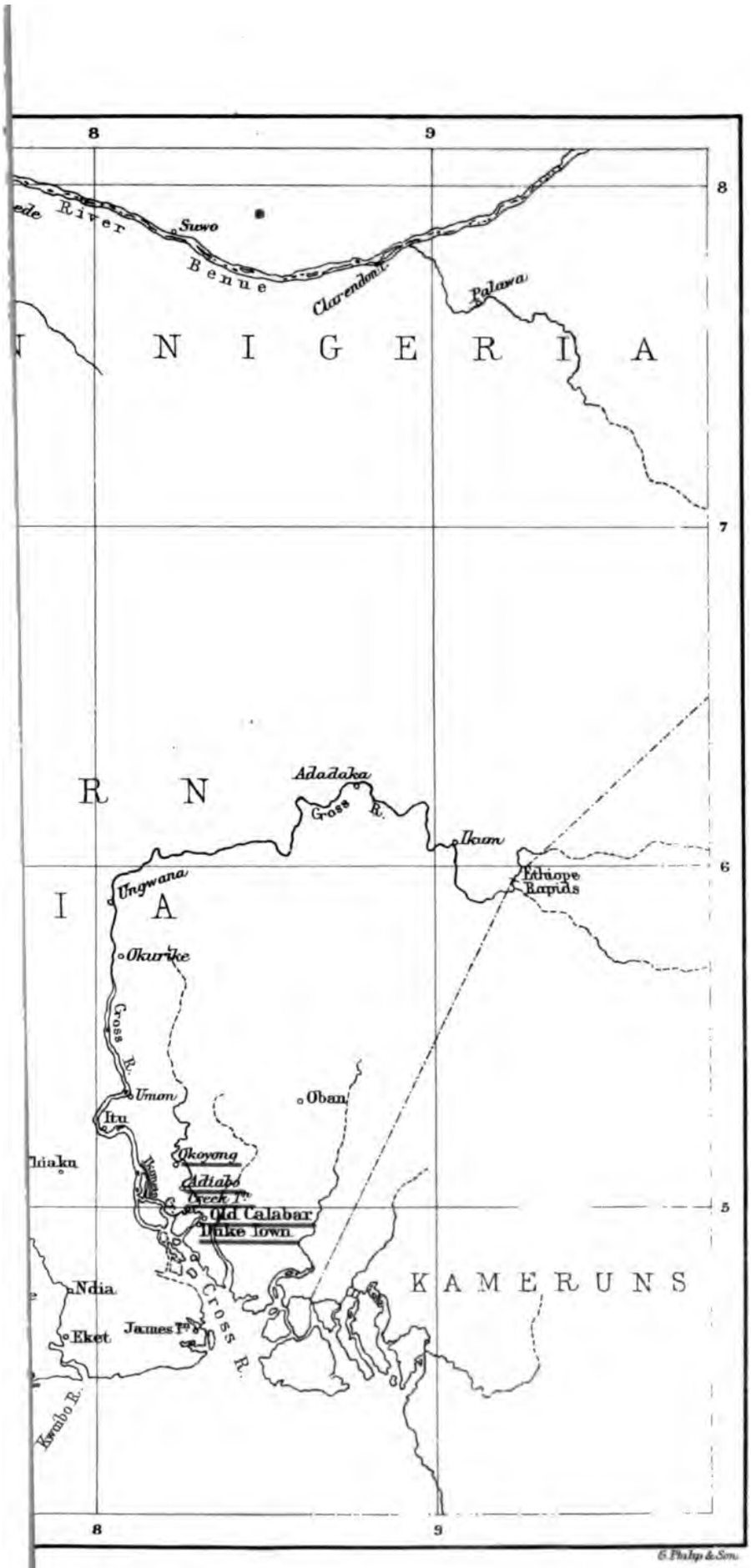
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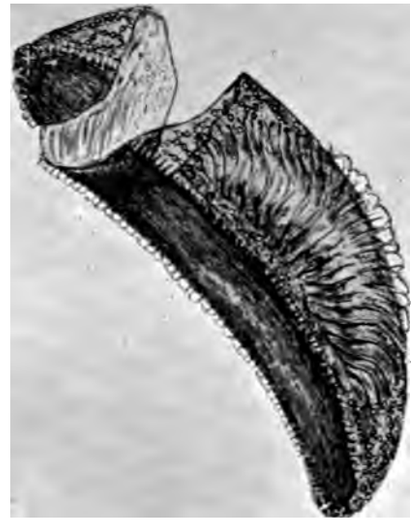
FIG. 1

FIG. 2

FIG. 3

FIG. 4



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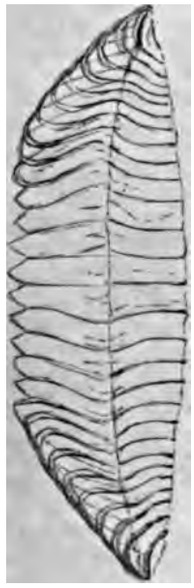

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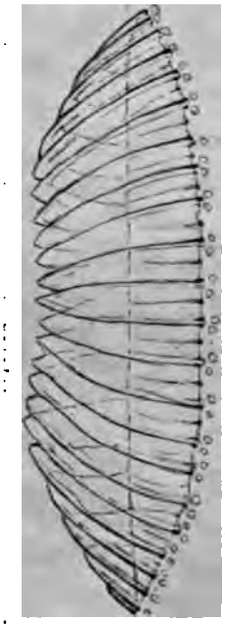

FIG. 7

FIG. 1. *Anopheles* ovum—superior surface
FIG. 2. „ „ —inferior „
FIG. 3. „ „ —side view
FIG. 4. *Culex* ovum
FIG. 5. *Anopheles* ovum—empty egg case
FIG. 6. The 'float'—upper surface
FIG. 7 „ „ —lower „

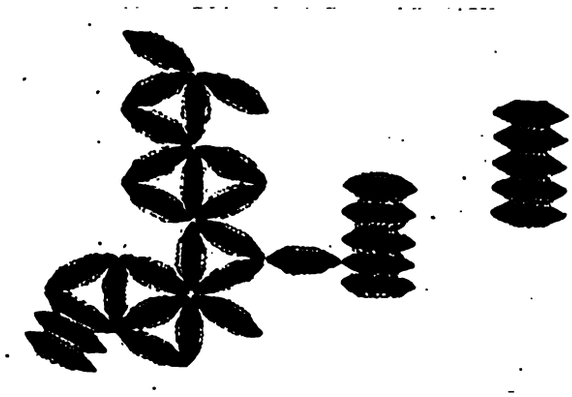

FIG. 1. The characteristic pattern in which *Anopheles* ova are deposited on the surface of water

FIG. 2. Showing larvae in several stages of their escape from the ova

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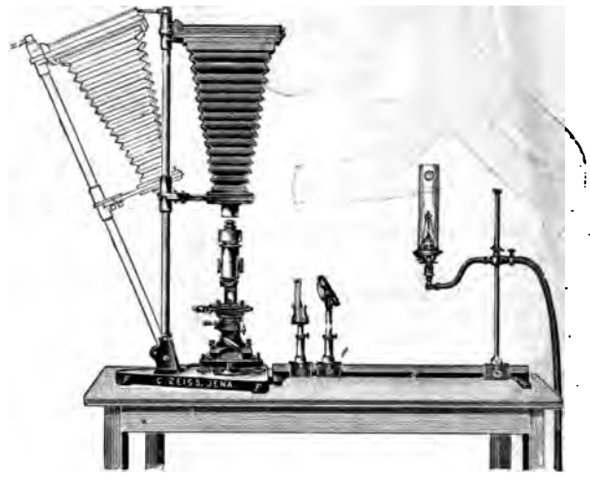

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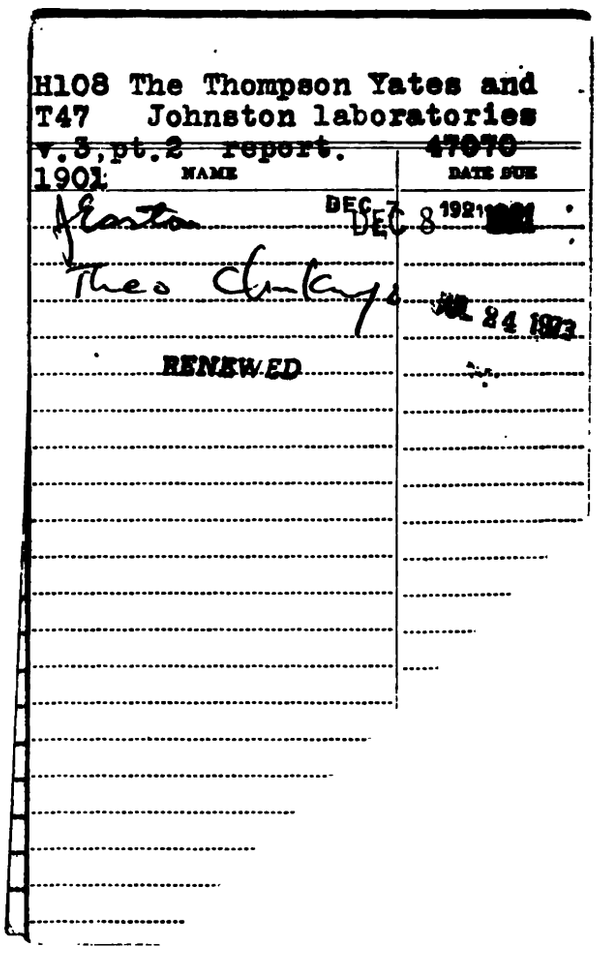
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